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Fish and Wildlife Service
Bureau of Commercial Fisheries
Galveston, Texas

ANNUAL REPORT
of the
GULF FISHERY INVESTIGATIONS
for the year ending June 30, 1958

George A. Rounsefell, Chief

This report constitutes part of the annual report of the Division of Biological Research and is distributed separately in the Gulf area as a service to members of the industry and other interested persons. The complete report of the Division will be distributed from Washington. Persons who do not regularly receive this full report may obtain copies by writing Fish and Wildlife Service, Washington 25, D. C.

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SUMMARY OF YEAR'S RESEARCH

George A. Rounsefell, Chief

The past year produced several important advances in our research. The new method of marking shrimp by colored vital stains was given field trials and at the close of the year was being successfully employed full scale in the Tortugas region. This method provides a mark that remains after shedding, thus furnishing reliable material for estimating growth and mortality.

During the autumn red tide outbreak in Florida, the first extensive attempt at control was made by spreading 105 tons of copper sulfate with crop-dusting airplanes. Although moderately successful, the method proved too expensive to use for controlling large outbreaks, and the organisms appear to be able to rebuild their abundance within a couple of weeks.

Studies of the life history of the white and brown shrimp in the inside waters show that they each reach a peak of abundance at a different season of the year. This sharply reduces the interspecific competition on the nursery grounds.

All of these and other topics are fully discussed under the various projects.

Gulf States Marine Fisheries Commission

The Gulf Fishery Investigations actively participated in the autumn semi-annual meeting of the Commission held at Mobile, Alabama, where in response to the Commission's queries concerning marine pond culture the Service proposed to cooperate with state efforts by conducting the necessary basic research under controlled conditions. A meeting of scientists under the sponsorship of the Commission was held in Ocean Springs, Mississippi in February, where working committees prepared reports on research results which were presented to the Commission at their April meeting in Clearwater, Florida.

The Commission passed, among other resolutions, one requesting the Service to employ Saltonstall-Kennedy funds for basic research on shrimp farming, and another suggesting that the Service expand the biological work on menhaden to take advantage of the opportunity to collect biological material on clupeoid fishes during the exploratory fishing cruises of the OREGON.

Engineering Projects

Most of the important fisheries of the Gulf are intimately connected with the shallow waters, marshes, and estuaries lying behind the barrier islands. The young of the white, the brown, and the pink shrimp enter these waters through the passes as larvae or postlarvae and grow very rapidly to a good size before emigrating seaward. The same applies to the young of the menhaden, mullet, tarpon, sea trout, and other species.

Because of the necessity for these nursery grounds the Service has been concerned lest engineering and industrial projects and development impair their value, if such developments are not adequately planned. The Gulf Fishery Investigations laboratory at Galveston has plans developed by the Galveston District Office of the Corps of Engineers for a seawater system with aquaria, tanks, and ponds to use in studying the effect of environmental changes, such as could occur on the marshes, on shrimp, menhaden, and other organisms using these shallow areas.

These plans call for pumping 1.39 cubic feet per second from a platform 1,200 feet out in the Gulf (depth of -8 feet) which connects with the seawall by a 3-foot steel catwalk on concrete pilings. The main seawater lines will be six-inch asbestos cement pipes in duplicate to allow for freshwater flushing and soaking to prevent fouling.

The seawater will first enter a 75,000 gallon storage and sedimentation tank. A portion of the water will be used to supply indoor aquaria (through plastic pipes) the length of the main laboratory building. Behind the main laboratory plans call for a battery of 16 small tanks (5 x 10 x 3 feet each) under a 45 x 65 foot greenhouse. Six large tanks 20 x 40 feet with a 6-foot depth will permit large scale experiments, and all water will be reused once in a 48-foot circular pool for pelagic fishes of large size.

The Service has also contracted with the Texas A. and M. Research Foundation to prepare detailed plans for studying the hydrography of the marshes, bayous, and adjacent portions of the Gulf of Mexico that may be affected by the proposed Mississippi River-Gulf Outlet Project for a sealevel canal connecting New Orleans directly with the Gulf of Mexico.

FACILITIES

Lawrence E. Wise, Administrative Officer

The Gulf Fishery Investigations' headquarters are located at Fort Crockett, Galveston, Texas. At the present time we occupy seven buildings, four of masonry construction and three of wood construction. The total area is approximately 400 feet long by 200 feet wide. In addition to these facilities we have an easement on the East Beach Lagoon at the present time being used for scientific experiments on ore dyke studies. The Gulf Fishery Investigations' headquarters boasts a well equipped analytical chemistry laboratory and a fishery library under the direction of an experienced Librarian.

The buildings were run down through lack of use and care prior to occupancy of the Fish and Wildlife Service. During this year considerable funds have been expended on rehabilitation -- installation of central heat and air conditioning in the Chemistry Laboratory and the main office building, remodeling for laboratory use and general electrical installation.

To make the buildings entirely safe considerable work will have to be done in painting, roof repairs and masonry repairs on the main buildings. The installation of air conditioning and filtered air in the chemistry laboratory has made possible accurate quantitative analyses for trace elements. In addition in the chemistry laboratory there has been constructed an insulated constant temperature instrument room allowing freedom from dirt and dust.

The red tide laboratory has been improved by the addition of constant temperature rooms, a light room and a germ free room. These improvements have materially augmented research and culturing of G. breve.

New offices have been established on the second floor, Laboratory A, for the Chief and Assistant Chief, and for housing the library.

The revised tide program has eliminated the use of a service airplane thereby eliminating an estimated expenditure in excess of \$12,000 per annum. This has been done by a cooperative agreement with the State of Florida who are extending their sampling program to allow our Florida Laboratory to restrict its area of operation and concentrate on ecological factors.

The Florida Laboratory has moved from Naples, Florida to St. Petersburg Beach, Florida in the latter part of August 1957. This move was necessary to make adequate building facilities available at nominal cost and to furnish a more suitable headquarters for operation. At the Florida Laboratory, St. Petersburg Beach, there has been installed an analytical chemistry laboratory which has resulted in considerable savings and obviated the necessity for shipment of water from Florida to Galveston for analyses. Analytical work of this nature is now being performed at the Florida Laboratory. The Florida Laboratory is headquarters for the vessel KINGFISH. New engines and other repairs this year have placed the vessel in first class operating condition. The vessel is manned by a Captain-Engineer (Master) and a Cook-Deckhand.

During the fiscal year Mr. Costello was transferred from Galveston to Coral Gables, Florida to work on Tortugas shrimp and has been furnished office space with the statistical branch of the Fish and Wildlife Service.

Public Relations

The Gulf Fishery Investigations' collection of specimens of marine life is at the present time the only one in Galveston. As a result numerous requests from the public schools, civic groups, boy scouts, girl scouts, and other interested individuals have been made to visit the Laboratory. During the past year over 400 visitors have viewed this collection and judging from expressions of appreciation the time was well spent. The laboratory has furnished exhibit material for such activities as March of Dimes, high school science fair, the Galveston Library, and boy scouts wildlife exhibit.

A series of eight talks on marine biology has been presented during the year by Mr. Edgar L. Arnold, Jr., Fishery Research Biologist on "Get Up Time", a television show originating in the Galveston Studio on KGUL-TV. Mr. Arnold also gave a lecture on biology in Brownsville, Texas to a vocational education group sponsored by the Texas Shrimp Association.

ORGANIZATION AND STAFF

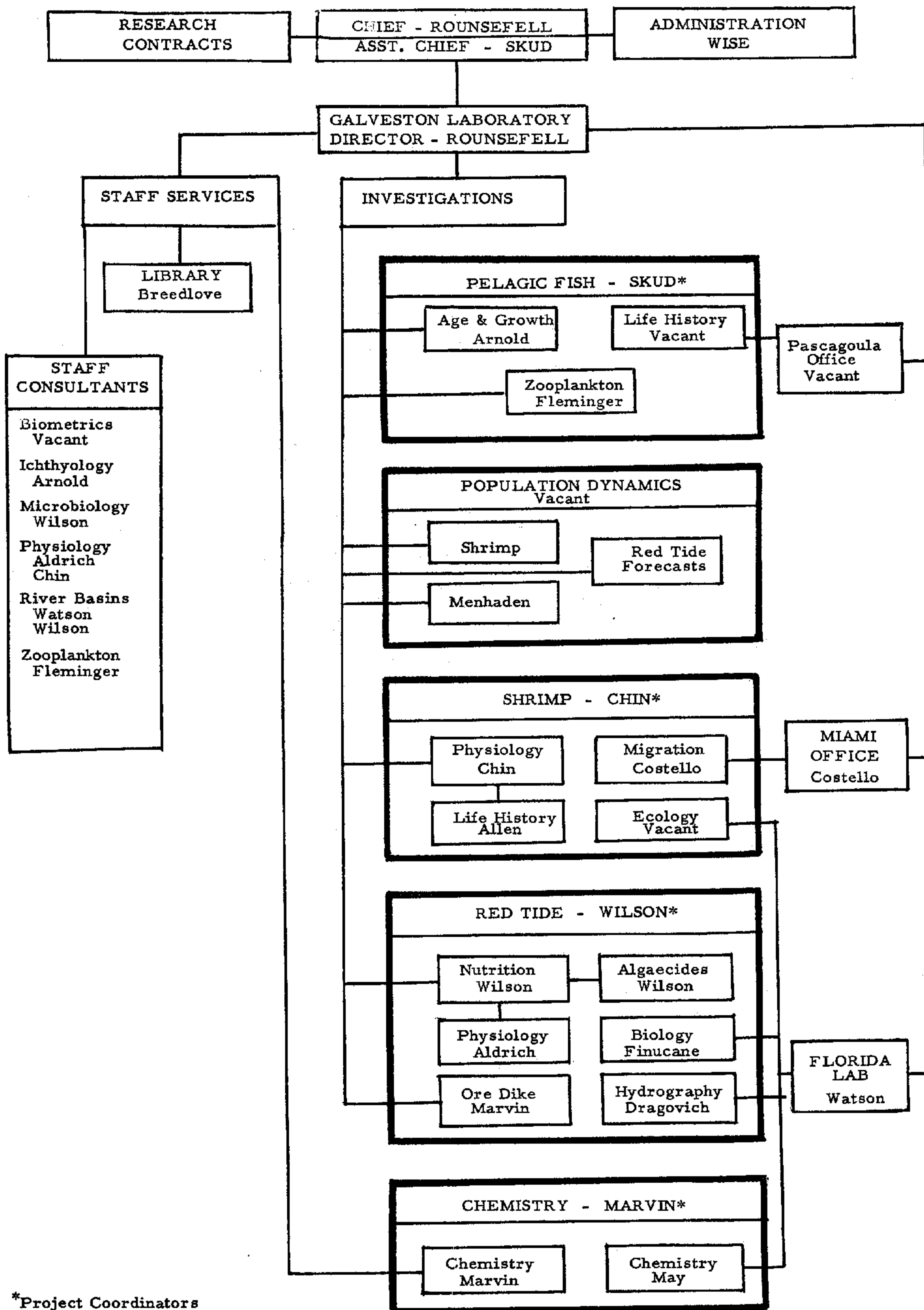
In line with the policy of the Bureau of Commercial Fisheries, the Gulf Fishery Investigations has increased its service to the fishing industry of the Gulf area during this past year. The former red tide substation at Naples, Florida has been moved to a new location in adequate quarters in St. Petersburg Beach. Provided with better facilities, including a chemistry laboratory, the program has been expanded to include work on shrimp and basic estuarine hydrography.

A field office was established at Miami, Florida, to study shrimp migration, growth, and mortality using the new technique of marking with colored dyes, and to maintain close liaison with the Marine Laboratory of the University of Miami, in their contract with the Service on the Tortugas pink shrimp fishery.

After the first of July biologists will be stationed at Pascagoula, Mississippi, to cooperate with the Service's Exploratory Fishing and Gear Research Laboratory in studying life histories of herring-like fishes taken by the research vessel OREGON.

The carrying on of research on different phases of similar problems at different locations has necessitated certain modifications in organization to provide for adequate liaison, coordination of programs, and necessary staff services, including consultation. For each major program a coordinator will tie it together wherever it is carried on. The present organization is shown in the diagram.

ORGANIZATION OF GULF FISHERY INVESTIGATIONS



*Project Coordinators

STAFF

GULF FISHERY INVESTIGATIONS

George A. Rounsefell, Chief
Bernard E. Skud, Assistant Chief (Pending)

GALVESTON LABORATORY (HEADQUARTERS - GALVESTON, TEXAS)

Research Staff

David V. Aldrich
Donald M. Allen
Edgar L. Arnold, Jr.
Edward Chin
Albert W. Collier, Jr. (Resigned January 31, 1958)
Abraham Fleminger
Anthony Inglis
Mary Elizabeth Jones (Resigned August 2, 1957)
Kenneth T. Marvin, Chemist
Sammy M. Ray
Theodore J. Starr (Resigned September 30, 1957)
Ray S. Wheeler
William B. Wilson
Zoula P. Zein-Eldin, Chemist

Technical Aids

Genevieve B. Adams
Kenneth N. Baxter
Roosevelt Boston (Resigned September 6, 1957)
Patricia A. Egan (Resigned January 10, 1958)
Jean A. Gates
George Higgins (Resigned August 23, 1957)
Melvin H. Hodge (Resigned April 4, 1958)
Maud Alice Kitchel
Larence M. Lansford
Imogene A. Sanderson
Charles W. Stroud (Resigned August 9, 1957)
Gilbert Zamora, Jr.

Approved for Release

Technical Assistants

Stella Breedlove, Librarian
James E. Forsythe (Resigned August 9, 1957)
Daniel Patlan, Drafting Aid

Administrative and Maintenance

Tidas C. Alcorn, Maintenanceman
Mary J. Atchison, Secretary (Resigned June 13, 1958)
Lucile A. Barlow, Clerk-typist
Corinna L. Denbo, Assistant Administrative Officer
Robert L. McMahon, Maintenanceman
Ruth W. Mullen, Secretary
Petronila C. Prado, Clerk-stenographer
Sarah M. Robison, Fiscal clerk
Joan M. Sanders, Fiscal clerk (Resigned November 1, 1957)
Esther E. Sell, Secretary
Glo S. Stover, Personnel clerk
Peter M. Villarreal, Maintenanceman
Lawrence E. Wise, Administrative Officer

Summer Aids

Walter E. Fosbert (Resigned August 30, 1957)
Robert G. Ghiselli (Resigned August 30, 1957)
Domingo R. Martinez
Raphael R. Proctor, Jr.
Harry D. Schwerdtfeger (Resigned August 30, 1957)
Gordon O. Stafford (Resigned August 30, 1957)
James A. Walmsley (Resigned August 30, 1957)

FLORIDA LABORATORY (ST. PETERSBURG BEACH, FLORIDA)

John E. Watson, Laboratory Head

Research Staff

Alexander Dragovich
John H. Finucane
Billie Z. May, Chemist

Technical Aids

William J. Becker (Resigned August 23, 1957)
Samuel B. Blackwood, Jr.
Carlton H. Furr, Jr. (Transferred to Statistical Branch, June, 1958)
McKinley W. Jambor
Lucius Johnson, Jr.
Charles R. LeBuff, Jr. (Resigned August 16, 1957)
Gilbert W. Moreau (Resigned August 30, 1957)
Albert L. Prince (Resigned November 29, 1957)

Technical Assistants

Laura M. Hermann, Administrative Assistant
Mary E. Hipple, Clerk-typist
Ruth V. LeBuff (Resigned August 30, 1957)
Louise G. Norwood (Transferred to Regional Office January 24, 1958)

Vessel KINGFISH

John D. McCormick, Captain-Engineer
James H. Levins, Sr., Cook-Deckhand

John E. Evans (Transferred to Regional Office April, 1958)

TORTUGAS SHRIMP PROJECT (CORAL GABLES, FLORIDA)

Thomas J. Costello, Jr., Project Leader

RED TIDE SYMPOSIUM

Since 1948 the Fish and Wildlife Service has been conducting research to determine the causes of outbreaks of Florida red tide, and especially to develop methods of prediction and control. It was deemed desirable to invite other scientists to review this decade of research, and to receive their comments, as it was hoped that such a discussion and appraisal would stimulate the research and aid greatly in formulating new programs.

A number of scientists invited by Director Donald L. McKernan and the research staff of the Gulf Fishery Investigations held the Symposium at the Galveston Laboratory on March 5-7, 1958.

The Advisory Group included:

- Robert M. Ingle--Director of Research, Florida State Board of Conservation
- Dr. Robert F. Hutton--St. Petersburg Laboratory of the Florida State Board of Conservation
- Frank Chew--Marine Laboratory, University of Miami, Florida
- Dr. E. F. Corcoran--Marine Laboratory, University of Miami, Florida
- Dr. James B. Lackey--University of Florida, Gainesville, Florida
- Dr. Gordon Gunter--Gulf Coast Research Laboratory, Ocean Springs, Mississippi
- Dr. Luigi Provasoli--Haskins Laboratories, New York
- Dr. Howard T. Odum--Institute of Marine Science, Port Aransas, Texas
- Dr. Carl H. Oppenheimer--Institute of Marine Science, Port Aransas, Texas
- Dr. K. M. Rae--Oceanography Department, Texas A. and M., College Station, Texas
- Albert Collier--Marine Laboratory, Texas A. and M., Galveston, Texas
- William W. Anderson--Chief, South Atlantic Fishery Investigations, U. S. Fish and Wildlife Service, Brunswick, Georgia
- Dr. Herbert W. Graham--Chief, North Atlantic Fishery Investigations, U. S. Fish and Wildlife Service, Woods Hole, Massachusetts
- Dr. Theodore R. Rice--Radiobiological Laboratory, U. S. Fish and Wildlife Service, Beaufort, North Carolina

Other visiting participants were:

Seton H. Thompson, Regional Director, Gulf and South Atlantic Region, U. S. Fish and Wildlife Service, St. Petersburg Beach, Florida

Dr. Albert L. Tester, Chief, Division of Biological Research, U. S. Fish and Wildlife Service, Washington, D. C.

Howard H. Eckles, Chief, Branch of Marine Fisheries, U. S. Fish and Wildlife Service, Washington, D. C.

Dr. J. Towne Conover, Institute of Marine Science, Port Aransas, Texas

Dr. Theodore J. Starr, University of Texas, Medical Branch, Galveston, Texas

The topics presented were:

1. Historical review of red tides and red tide research.
2. Geographical distribution of the red tide organism.
3. Hydrographic studies of the Florida west coast.
4. Life forms of the red tide organism, G. breve.
5. Laboratory studies of growth requirements of G. breve.
6. Toxicity of dinoflagellates and bacteria.
7. Control tests of copper sulfate and other algacides.
8. Testing of copper ore for control.
9. Forecasting of red tide outbreaks.

The afternoon of the third day was reserved for a meeting of the Advisory Group, each of whom presented his comments on the work to date and made suggestions for future studies.

As a result of this Symposium the staff has drawn up a revised program of research that we hope will speed our understanding of the factors underlying the overblooming of the red tide organism.



ECOLOGY OF SHRIMP

Donald M. Allen, Project Leader

The commercial shrimp catch in the Gulf of Mexico is comprised of three main species: the brown shrimp, Penaeus aztecus, the pink shrimp, Penaeus duorarum, and the white shrimp, Penaeus setiferus.

The white shrimp, the only one of the species with a well known life history, spawn in the open waters of the Gulf. The demersal eggs hatch into planktonic larvae which move towards the coast. Before reaching shore, the larvae develop into Post-larvae, which resemble the adults. The post-larvae, after entering the passes and arriving in the shallow, brackish inside waters, or "nursery grounds", settle to the bottom and grow rapidly. As the young shrimp grow larger and become mature, they gradually move out through the passes into the open waters of the Gulf, and return to the spawning grounds. The life cycle apparently takes a year, although indications are that some white shrimp may live for one and a half years.

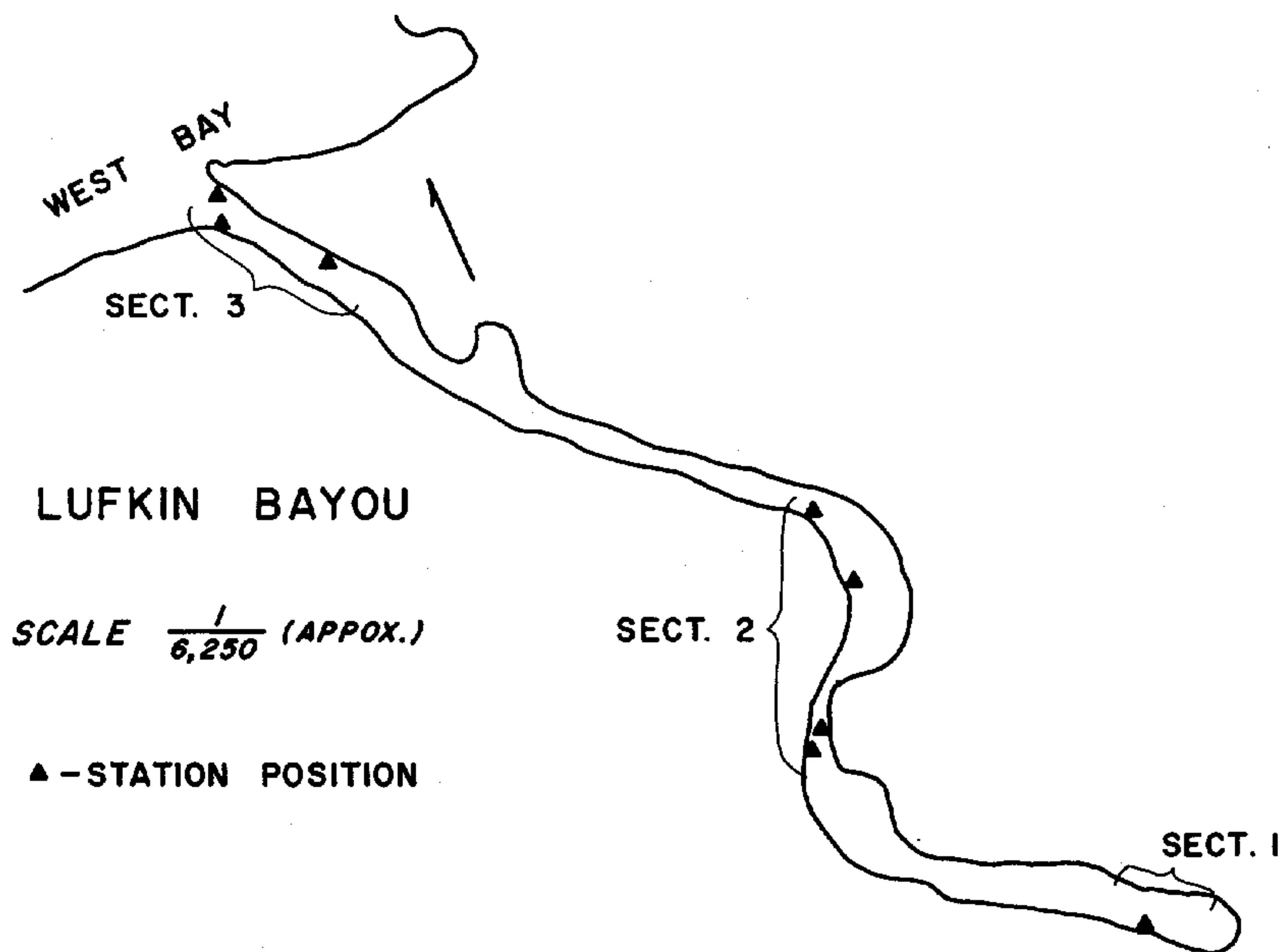
Since the period spent on the nursery grounds appears to be a particularly important phase in their life history, the shrimp research program has been designed to study:

1. The shrimp species composition in the inside waters to determine the overlap in space and time between species so as to evaluate the competition for nursery areas.
2. The distribution of the various species of shrimp within the inside waters in relation to physical and biological factors for the same reason.
3. The interrelationships of shrimp with the associated fauna. Not only is it of importance to study the community relationships within the estuary, but also to determine what species inhabit these nursery areas.
4. Methods and speed of inshore movement of young and post-larval shrimp.

Competition for Nursery Areas

It is well known that young of pink, brown and white shrimp all utilize the nursery grounds, and may exist in approximately the same

portion of the estuaries. For this reason it has been postulated that competition between the young in the nursery areas may be one factor controlling the seasonal or yearly abundance of the larger shrimp of each species.



Detail map of Lufkin Bayou (See figure in "Bait Shrimp Fishery" for orientation).

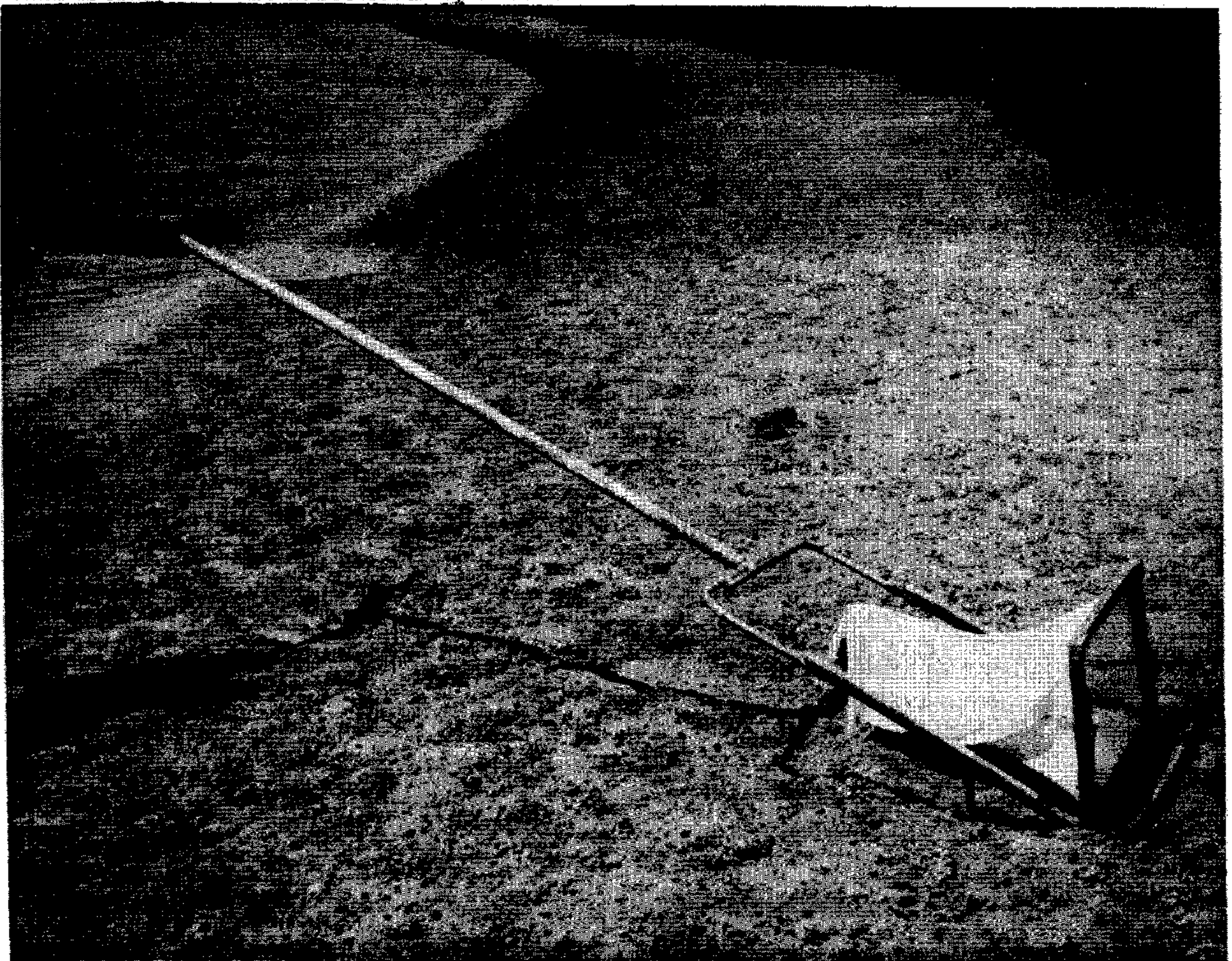
To investigate shrimp intraspecific competition within a nursery area, Lufkin Bayou, a small natural inlet indenting the marshy shore on the bay side of Galveston Island, Texas, was chosen, since it appeared to be physically typical of many of the local bayous. This bayou is approximately one mile in length and averages about 80 feet in width. The depth averages less than two feet at mean low tide, although depths up to six feet occur near the mouth. The substrate is typically muddy sand, and the dominant vegetation on the shores is salt-cord grass (Spartina alterniflora). During the period observed, May to December, 1957,

salinities ranged from 14.4 to 39.4 parts per thousand and water temperatures ranged from 10.2 to 37.6° C. Undoubtedly, much lower temperatures occurred during winter periods of low water associated with cold fronts.

Only brown and white shrimp were found in the bayou during the period of study, although a few pinks are also caught in the Galveston locality.

Insofar as possible, sampling stations were representative of the more obvious physical situations of the bayou, namely, type of bottom, type of vegetation, and distance from the mouth of the bayou.

In Lufkin Bayou the young shrimp generally concentrated along the shores in areas of stiff salt-cord grass where it is difficult to operate a beach seine. Although a pushnet used by Florida bait shrimp



Pushnet designed for sampling in stiff vegetation.

fishermen is superior to the seine for collecting in rooted vegetation, this net apparently operates most efficiently in vegetation which is less rigid than salt-cord grass. Since it appeared that no existing gear was satisfactory for our sampling program, a new type pushnet was developed, designed to yield quantitative samples while being operated by one man in stiff rooted vegetation in depths of from 3 to 18 inches. In actual use, the pushnet was pushed at a standard pace for a distance of 50 feet at each station. With this net we caught penaeid shrimp ranging in length from 6 to 85 mm in beds of partially inundated salt-cord grass as well as in shallow water devoid of vegetation. Shrimp larger than 85 mm probably did not commonly occur in these shallow water stations, since they were never seen leaping to avoid the net as were the smaller shrimp.

Attempts to sample the "deeper" water of the bayou were generally unsuccessful, since the soft mud bottom hampered use of the pushnet, and the shallowness of the water usually prevented employment of sampling gear which required the use of an outboard motor.

Specimens of brown and white shrimp under 18 mm in length are extremely difficult to tell apart. Therefore, in discussing the data we have not included any shrimp under 18 mm in length.

Seasonal Distribution

Brown Shrimp (Penaeus aztecus)

Brown shrimp were abundant on May 15 when sampling was begun in Lufkin Bayou. Furthermore, incidental sampling conducted in the Galveston locality earlier in the year indicates that post-larval browns entered the bayous as early as February.

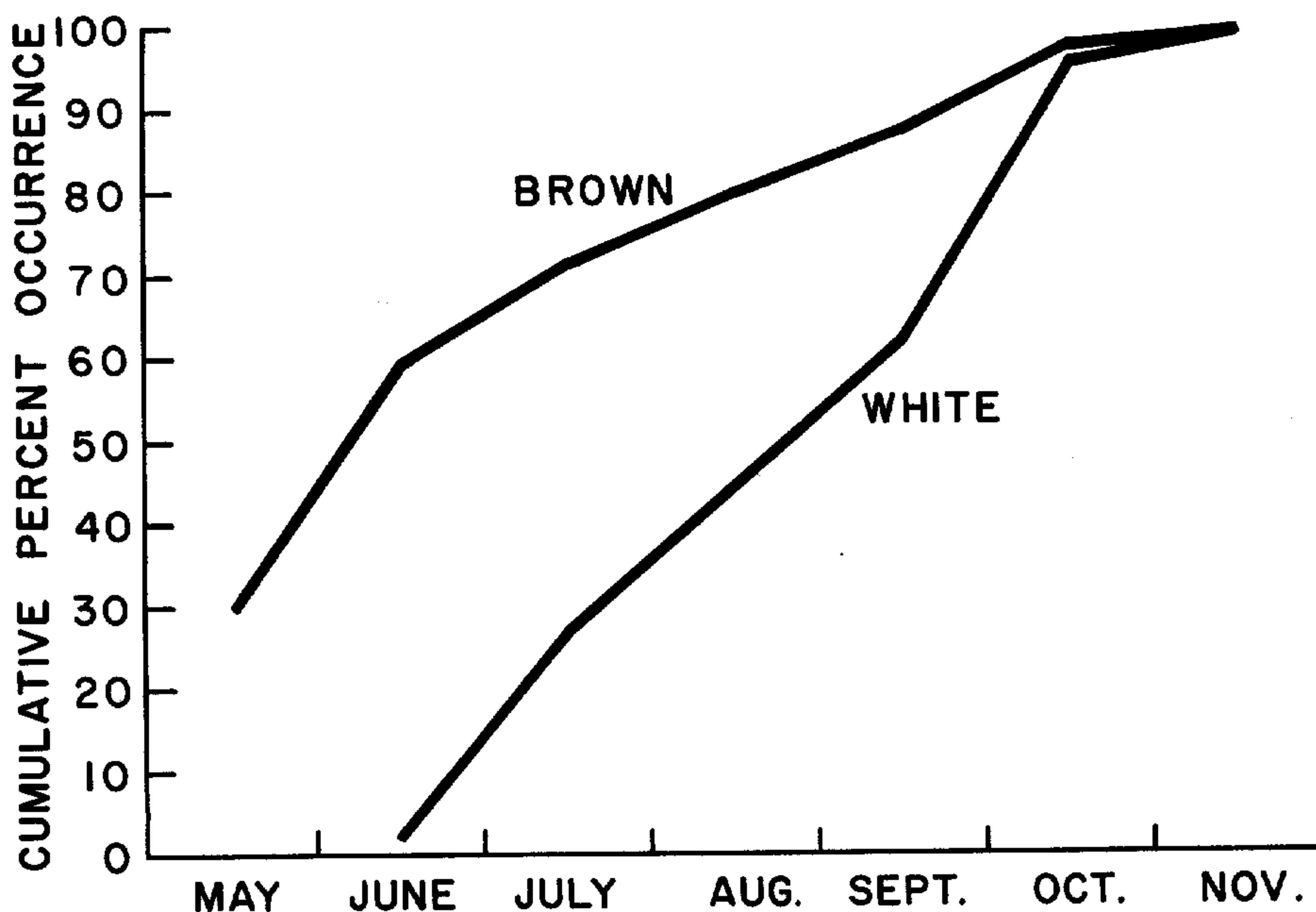
The young browns were most abundant in the latter part of May and the first part of June; by the middle of June their numbers had diminished. They were common until a small peak was reached in early October; and then remained common through early November.

White Shrimp (Penaeus setiferus)

The first white shrimp sufficiently large to identify appeared in our samples on June 6, and probably entered the bayou as post-larvae in the middle of May.

White shrimp reached a peak during the last part of July, declined but were common throughout the remainder of the summer

and fall, and achieved their maximum abundance during the first part of October. They remained common into early November.

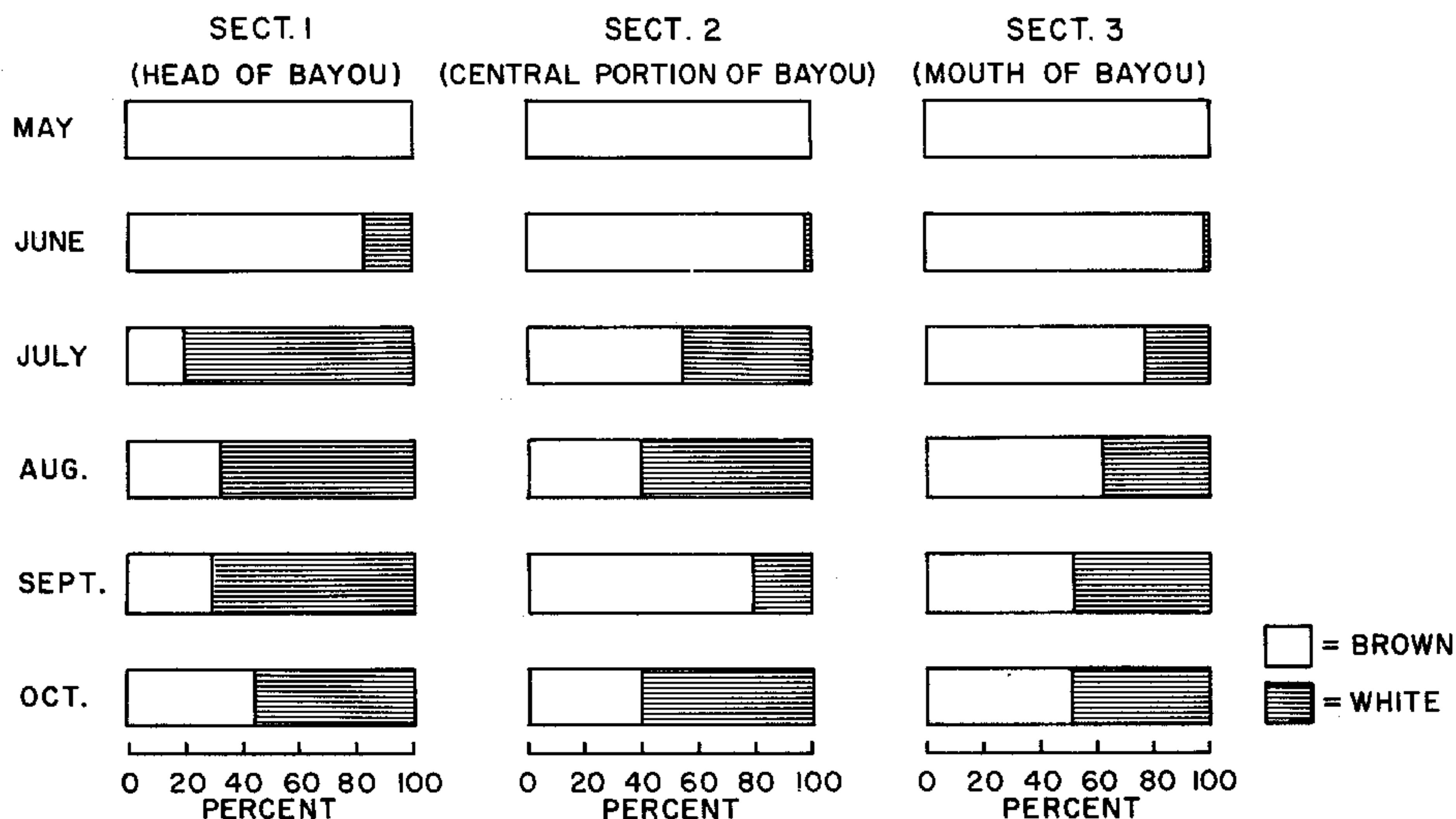


Cumulative abundance of brown *P. aztecus* and white shrimp *P. setiferus* by months in Lufkin Bayou.

The beginning of post-larval entry of each species onto the nursery grounds was widely separated, and the greatest proportion of browns had departed from the bayou more than two weeks before whites became common. It appears, therefore, that no serious competition existed between the two species in Lufkin Bayou.

Distribution within the bayou

Once white shrimp became common in the bayou, both species were found associated at all stations, however, white shrimp were most common near the head of the bayou, whereas the brown shrimp were dominant near the mouth. Dominance of either species in the central portion of the bayou appeared to be transitory as shown in the figure.

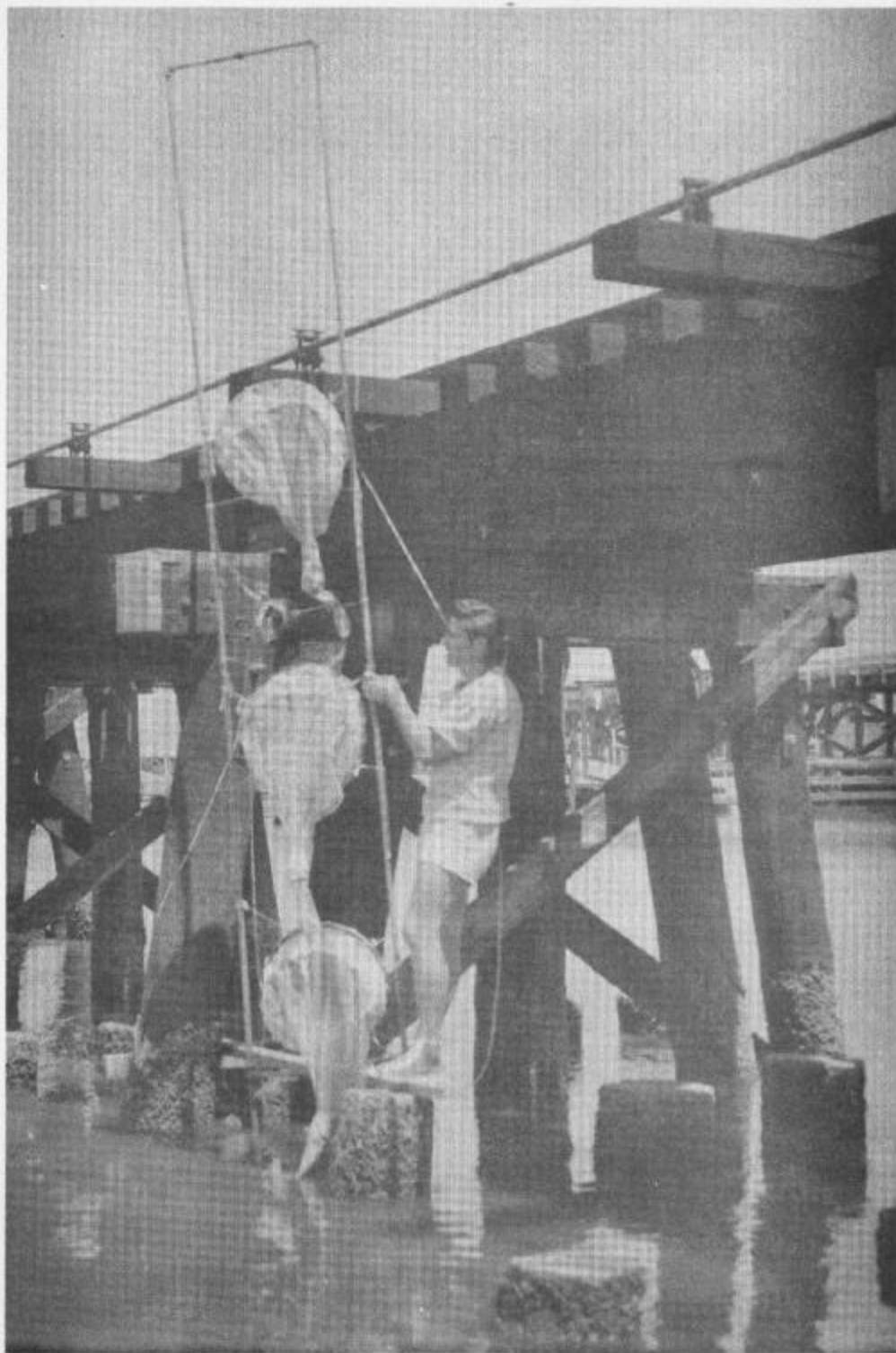


Relative abundance of brown P. aztecus and white shrimp P. setiferus by months and bayou sections.

Some investigators have noted that young whites seem to prefer lower salinities than young browns. It does not seem likely that salinity was the determining factor in this particular instance. The average monthly salinity at the head of the bayou never differed more than 2.3 parts per thousand from that at the mouth of the bayou; and for four out of the six months the salinity at the head was higher. Although the possible importance of salinity in the relative distribution of these two species cannot be discounted; the possibility exists that under certain conditions young white shrimp exist in areas which are more remote

from the ocean than browns for reasons other than salinity.

The work at Lufkin Bayou had to be terminated due to dredging and filling of adjacent land for real estate purposes. Therefore, the shrimp field studies have been shifted to Clear Lake, about 30 miles from the Galveston laboratory.



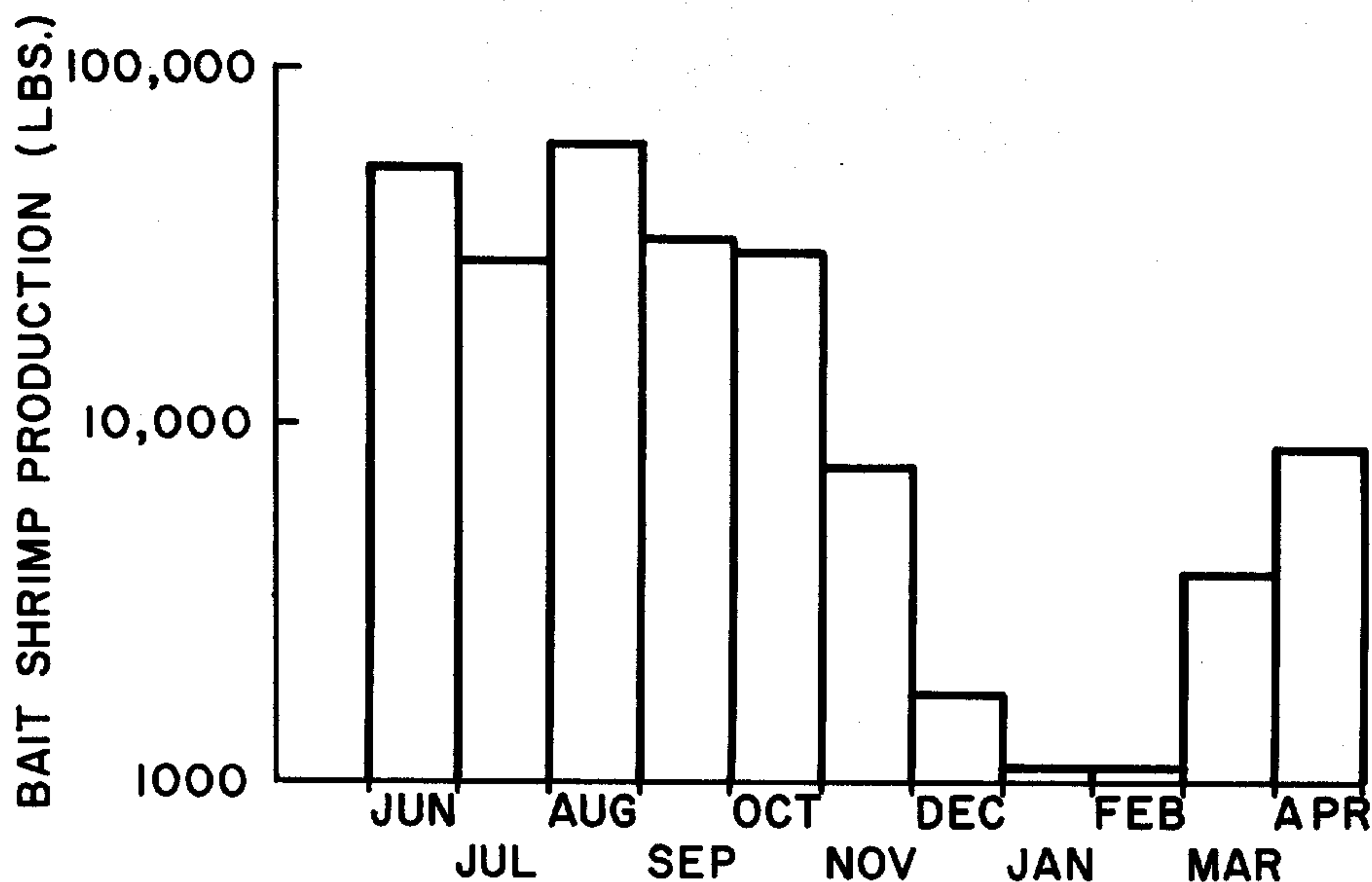
Plankton nets for sampling post-larval shrimp at different levels.

THE BAIT SHRIMP FISHERY OF GALVESTON BAY

Edward Chin, Project Coordinator

Several species of commercially important shrimp are used as bait by sport fishermen in the Gulf of Mexico, giving rise to a bait shrimp industry of considerable importance in certain localities. In June, 1957, a study was initiated to determine the magnitude of the bait shrimp fishery in Greater Galveston Bay and the size and species of shrimp that entered into the fishery.

As is the case in many fisheries, the collection of statistics proved to be a formidable problem. A preliminary survey in November, 1956, showed about 200 bait stands in the area distributed as shown in the accompanying figure. Due to the unstable and seasonal nature of the industry and the relatively small investment required to start a bait stand, the number of stands actually in operation fluctuates almost



Bait shrimp production in Galveston Bay area, 1957-1958

daily. During the course of the study, the number of operators that did the actual fishing ranged between 50 and 75. Nevertheless, they were so widely distributed that the two statistical agents were seldom able to interview each dealer oftener than every two or three days. Since few dealers keep records, either as to amount caught or composition of the catch, information collected during an interview was generally based on memory and estimates by the dealer. There was also a natural reluctance on the part of many dealers to disclose catch statistics. The figures obtained, thus, are considered minimal.

The total production of bait shrimp for the eleven month period from June, 1957, to April, 1958, amounted to about 225,000 pounds with a retail value of about \$250,000. Almost all the shrimp were obtained within Galveston Bay. Less than 5,000 pounds were brought in from the Sabine area, about 60 miles from Galveston, from December to April. As shown in the accompanying figure, the bait shrimp industry is at its height during the summer and is practically nonexistent in the winter.

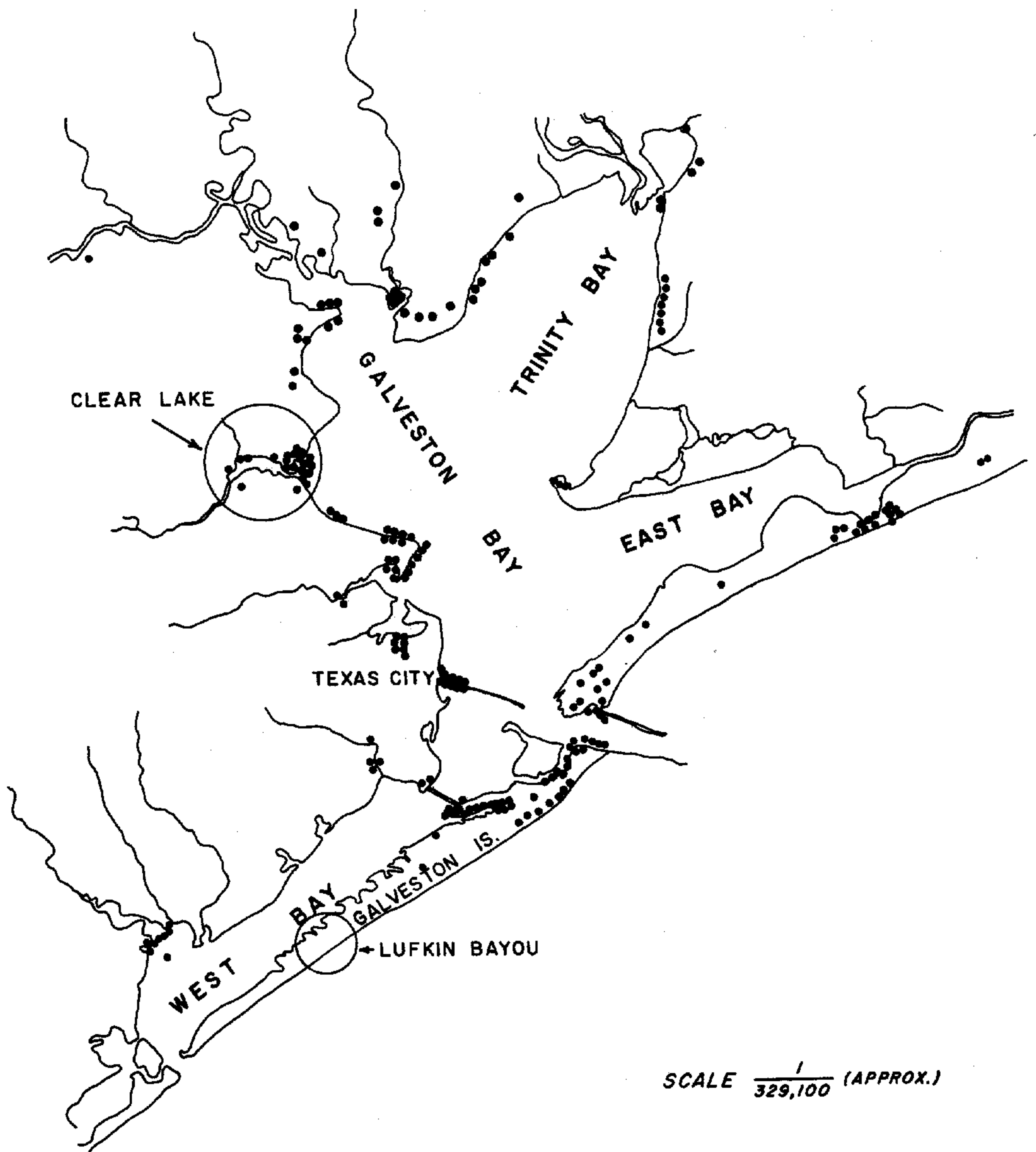
Unfortunately, Hurricanes Audrey and Bertha caused a great deal of damage to the bait industry and the production for the month of July was considerably below normal.

The production by the bait shrimp fishery is interesting when compared with that by the commercial fishery in the same area. In June, less than 3,600 pounds of shrimp were reported from the commercial fishery compared to over 51,000 pounds from the bait fishery. In July, despite the damage wrought by two hurricanes, 28,000 pounds were landed by the bait fishery. No commercial shrimp were landed in July, due partially to the closing of Galveston Bay to commercial ~~shrimping from July 15 to August 31.~~

Local fishermen interpret the regulations as permitting fishing on August 31, and as a result, almost 47,000 pounds of shrimp were produced by the commercial fishery on August 31. In comparison, the bait fishery produced over 60,000 pounds in August.

From September, the commercial fishery far outstrips the bait fishery. Nevertheless, it is interesting that for three months (June, July, August) and possibly four (the month of May gives every indication of being a productive month for the bait fishery), the commercial catch is exceeded considerably by the catch of bait shrimp.

Since no records on the composition of the catch were available, samples were purchased periodically from dealers selected at random. In almost cases, we were able to obtain samples of shrimp that had been caught the same day in a well-defined area. All shrimp were identified and weighed on a direct-reading balance. Total weight was



Location of bait shrimp stands in Galveston Bay area, 1957.

used as a measure of size because of the rapidity with which the shrimp could be weighed.

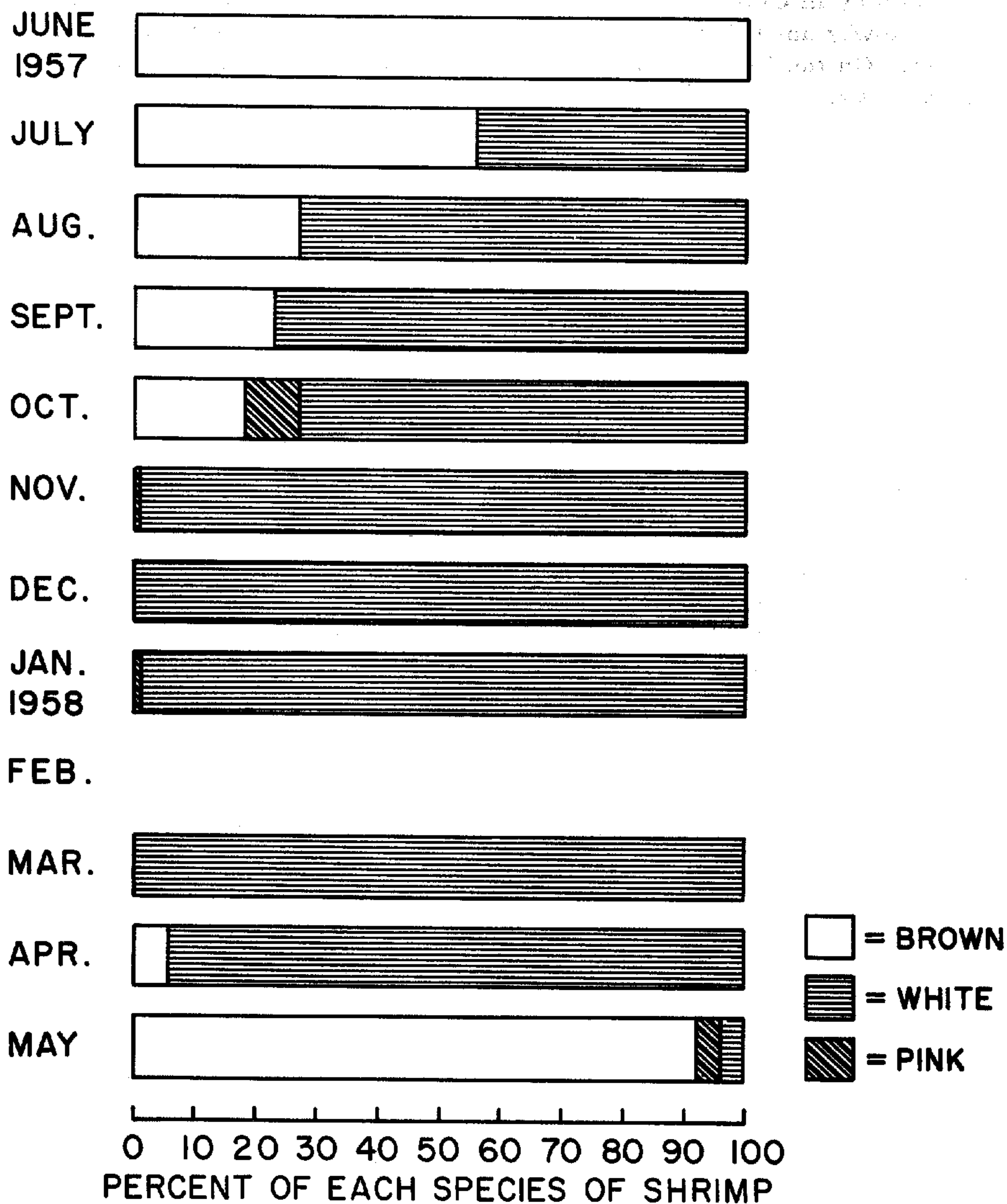
The species composition of the samples, combined by months is shown in the accompanying figure. The brown shrimp (Penaeus aztecus) dominated the bait fishery at the beginning of the study in June. The white shrimp (Penaeus setiferus) first appeared in July. As the season progressed, the white shrimp became increasingly important and by October, made up over 80 per cent of the catch. By November, the brown shrimp had virtually disappeared from the fishery. During the winter, white shrimp were scarce in the bay, but some were always present. Brown shrimp were completely absent until April. In May, white shrimp began to drop out of the picture as brown shrimp resumed a dominant role in the catch. Thus, on the basis of this study, there appear to be few problems of competition between the white and brown shrimp inside the bays.

The appearance of pink shrimp (Penaeus duorarum) in the bait fishery was sporadic and relatively insignificant.

The brown shrimp ranged in weight from one to eleven grams. There was an almost static mode at 2-3 grams indicating a long spawning season and continual migration into and out of the bay. Furthermore, it appears that brown shrimp leave the bays at a considerably smaller size than the white shrimp. Large browns, unlike whites, were not found in the bays at any time.

The white shrimp varied considerably in weight, ranging from one to forty grams, depending on the time of year. From the weight frequencies of the samples of shrimp taken inside the bay, three distinct groups of white shrimp could be detected. The first group, appeared in the fishery at about 2 grams and grew rapidly to 27 grams in September. In October, this group moved outside the bay, presumably on their way to the offshore spawning grounds. At this time the shrimp were about 36 grams.

In August and September, a second group of small white shrimp, about 2 grams, appeared in the fishery. These shrimp grew rapidly until October, at which time they were about 12 grams. They overwintered in the bay growing to about 20 grams in March. In April, this group also moved out of the bay, giving rise to a brief flurry of commercial shrimping just off the beaches. We were unable to sample the fishery at this time, but the commercial statistics from that area reported about 18,000 pounds of white shrimp, of which almost 16,000 pounds were 31-35 count, heads-off.



Percentage species composition of bait shrimp caught in Galveston Bay area.

A third group of white shrimp, also about 2 grams, appeared in the fishery in October and overwintered in the bay. These shrimp grew very slowly until April at which time they disappeared with the second group. On the basis of the available information, the fate of this group is unknown.

TORTUGAS SHRIMP FISHERY

Clarence P. Idyll

University of Miami Marine Laboratory

(Contract No. 14-17-008-7)

In September 1957 a study was outlined to gather needed biological information on the commercially important Tortugas pink shrimp fishery. Two preliminary sampling trips were made by E. L. Arnold and David V. Aldrich of the Fish and Wildlife Service staff. Following this initial work, a contract was negotiated with the University of Miami Marine Laboratory to carry on the study of the Tortugas fishery.

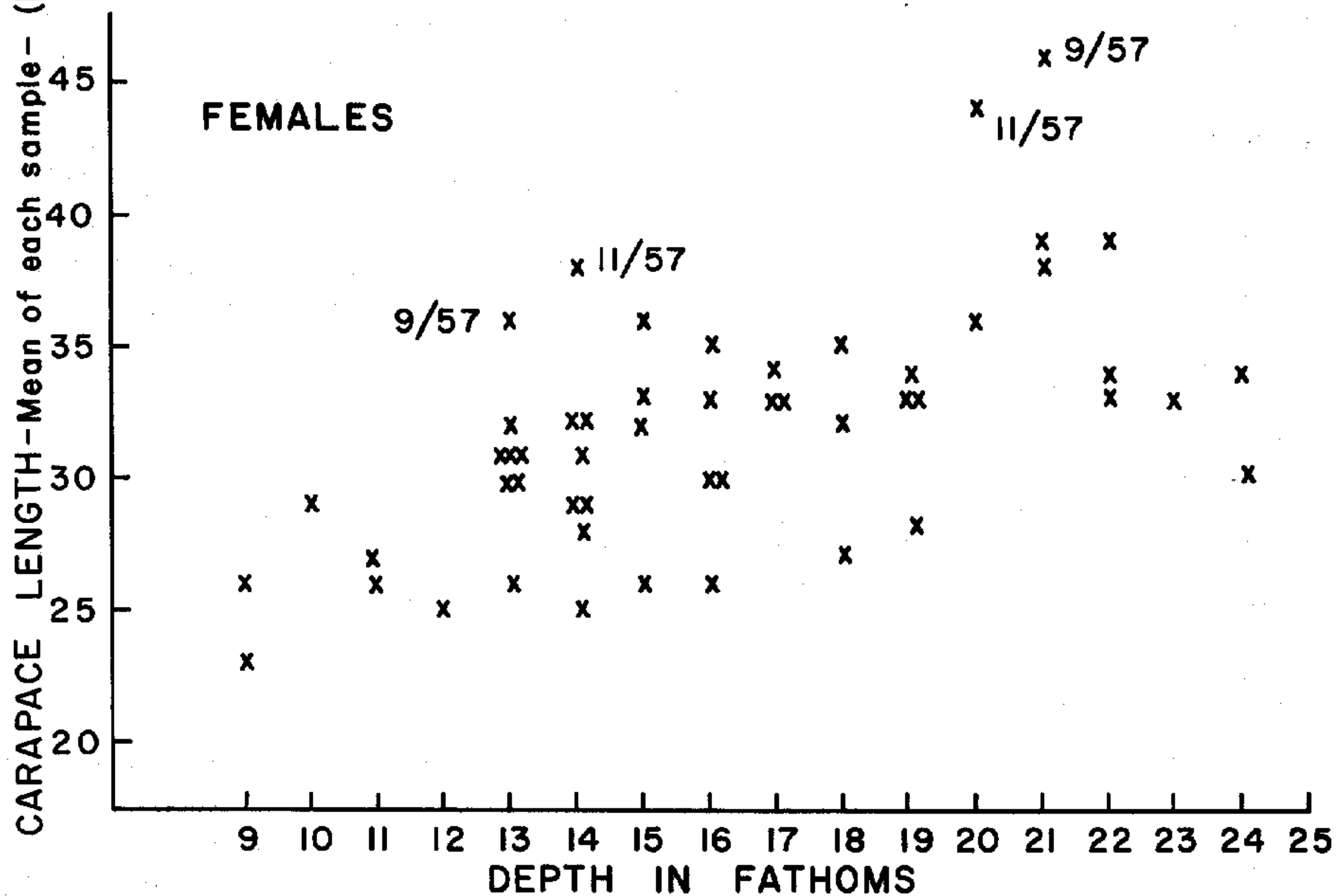
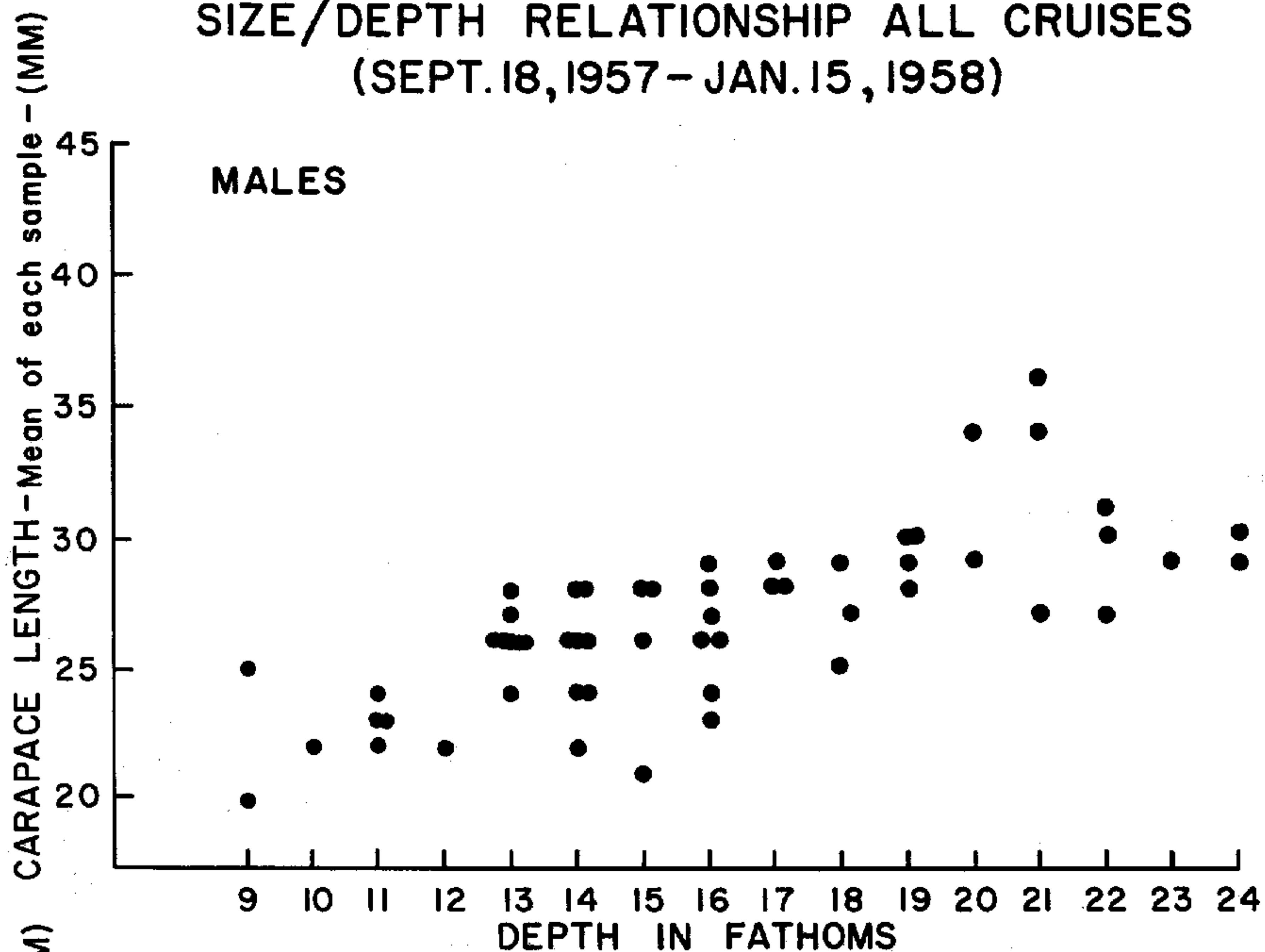
The contract specified two objectives of the "sea sampling" program to be given primary consideration: (1) Study the relationship of the size of Tortugas pink shrimp to the depth of capture; (2) Learn the importance and magnitude of discards of undersized shrimp at sea. These aspects of the fishery were studied first since a good deal of controversy had arisen over the reported waste of a considerable percentage of the catch. Also, as a basis for management, it is of obvious importance to learn if a clear-cut relationship exists between the size of shrimp and the depth of capture. Work formally began under the contract October 15, 1957, and the study to date has yielded useful information. The figure summarizes data on the relationship of size of pink shrimp to depth of capture for the period September 18, 1957 to January 15, 1958. A clear trend toward larger shrimp with increasing depth is shown. It is also interesting to note that the increase in size is related to distance from land without regard to depth.

A comprehensive view of the size depth relationship which exists is shown in another figure. This is drawn from samples of female pinks taken in 73 drags from October 1957 to February 1958 and illustrates the size distribution as it relates to depth contours in the Tortugas fishing area. (Note the South East Corner of this figure is approximately 14 nautical miles from Key West.)

Shrimp Discard

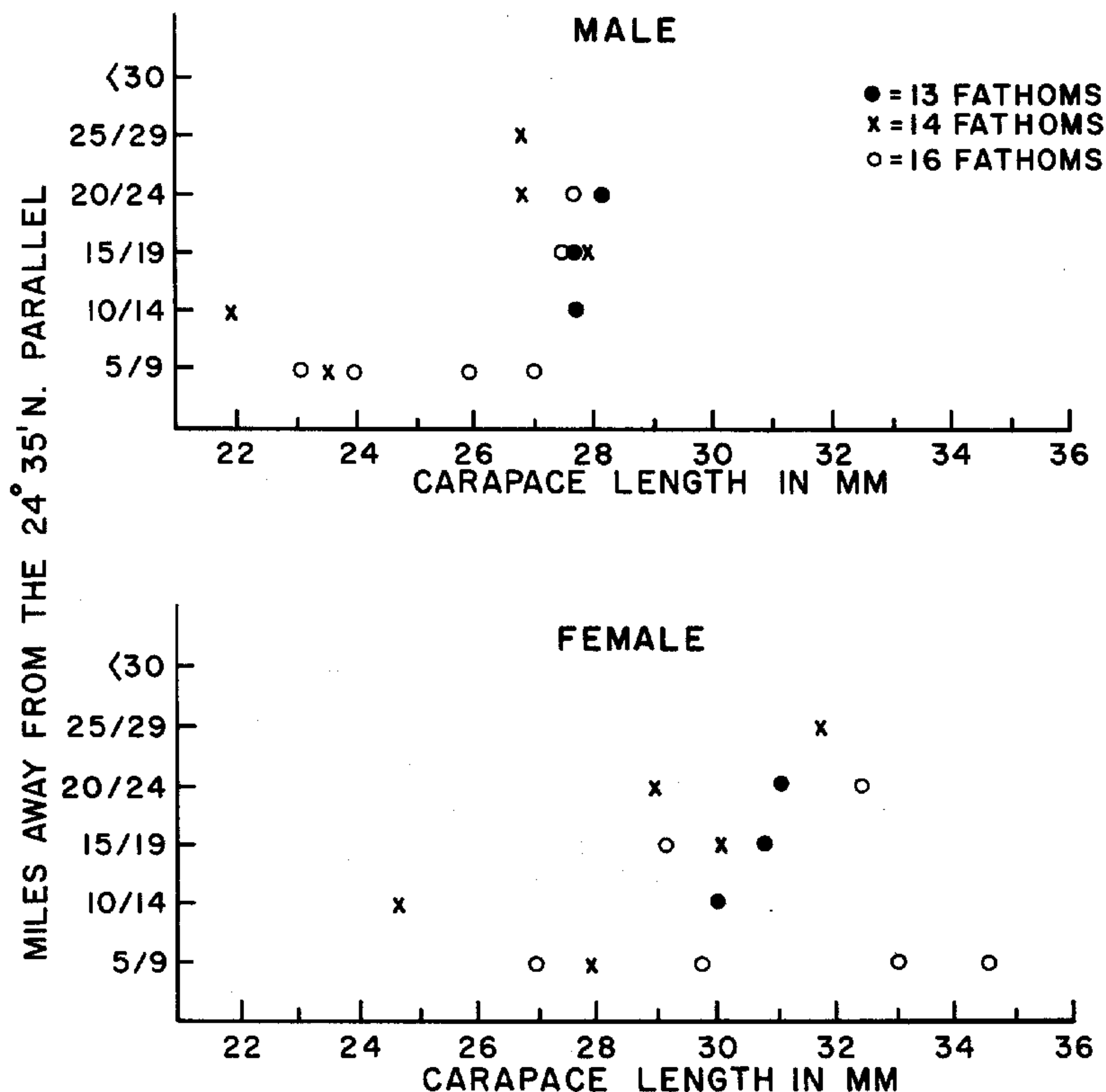
Observations of shrimp discards were made throughout the seven month period of contract work to date. In summary it is fair to say that the importance of discards appears to have been greatly exaggerated. In addition, many of the smaller shrimp which may have been discarded in the past are now being marketed at a good price.

SIZE/DEPTH RELATIONSHIP ALL CRUISES (SEPT. 18, 1957 - JAN. 15, 1958)



Size-depth relation of pink shrimp on Tortugas grounds.

SIZE DISTRIBUTION IN RELATION TO DISTANCE FROM LAND



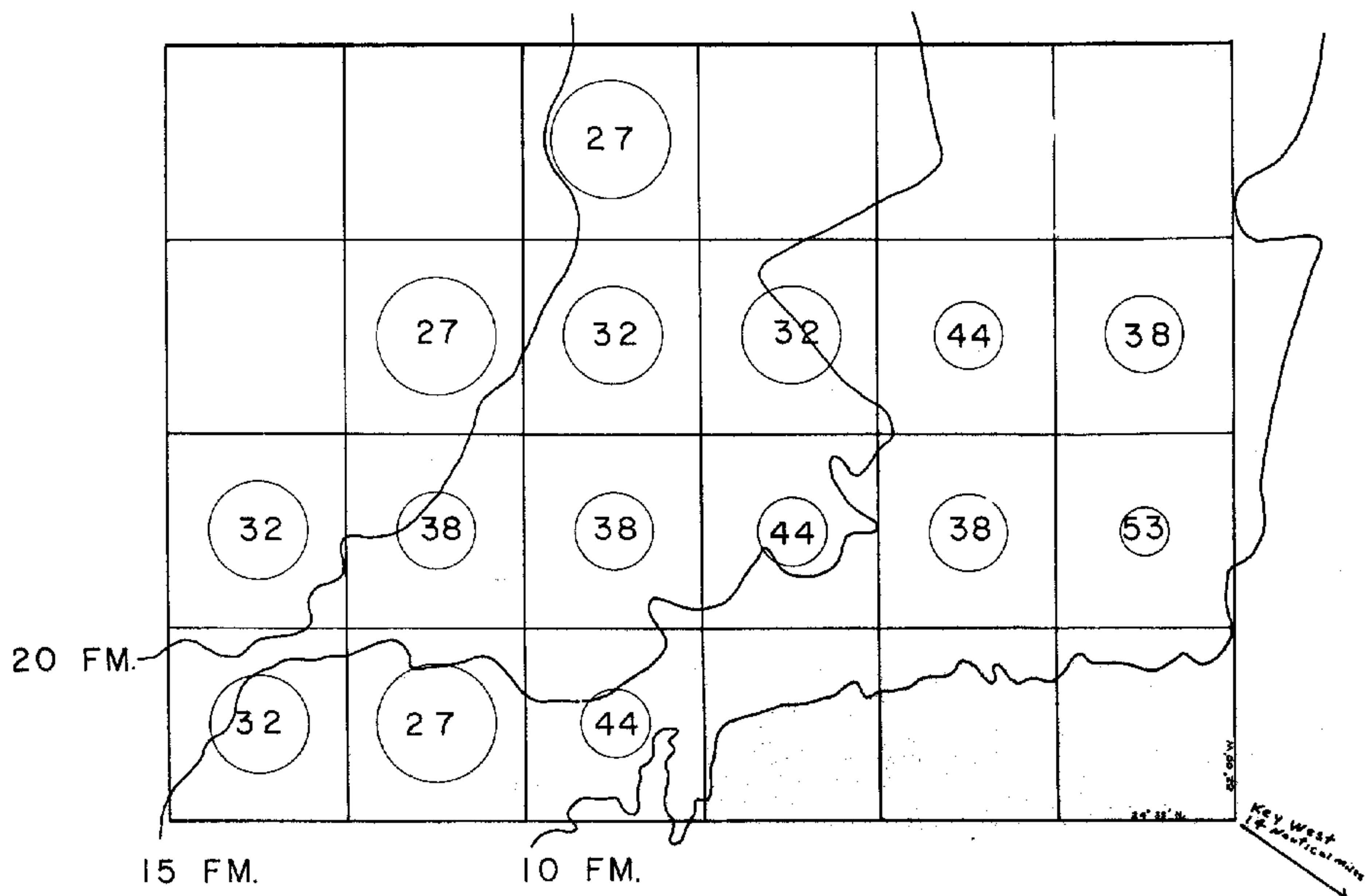
Size of pink shrimp in relation to distance from land in Tortugas fishery.

Tortugas Fleet Activities

Observations of the Tortugas fleet's activities including movement and size has added valuable information which will contribute toward understanding of this fishery. The observations, made from aboard the chartered vessel CAPTAIN MACK and by use of chartered aircraft, indicate this fleet has ranged from 388 to 658 boats during a seven month period. The area of heaviest fishing pressure is located 7 nautical miles northwest of Wreck Buoy along a line to the west to a point north of new grounds shoal.

SIZE DISTRIBUTION OF TORTUGAS PINK SHRIMP

FEMALES

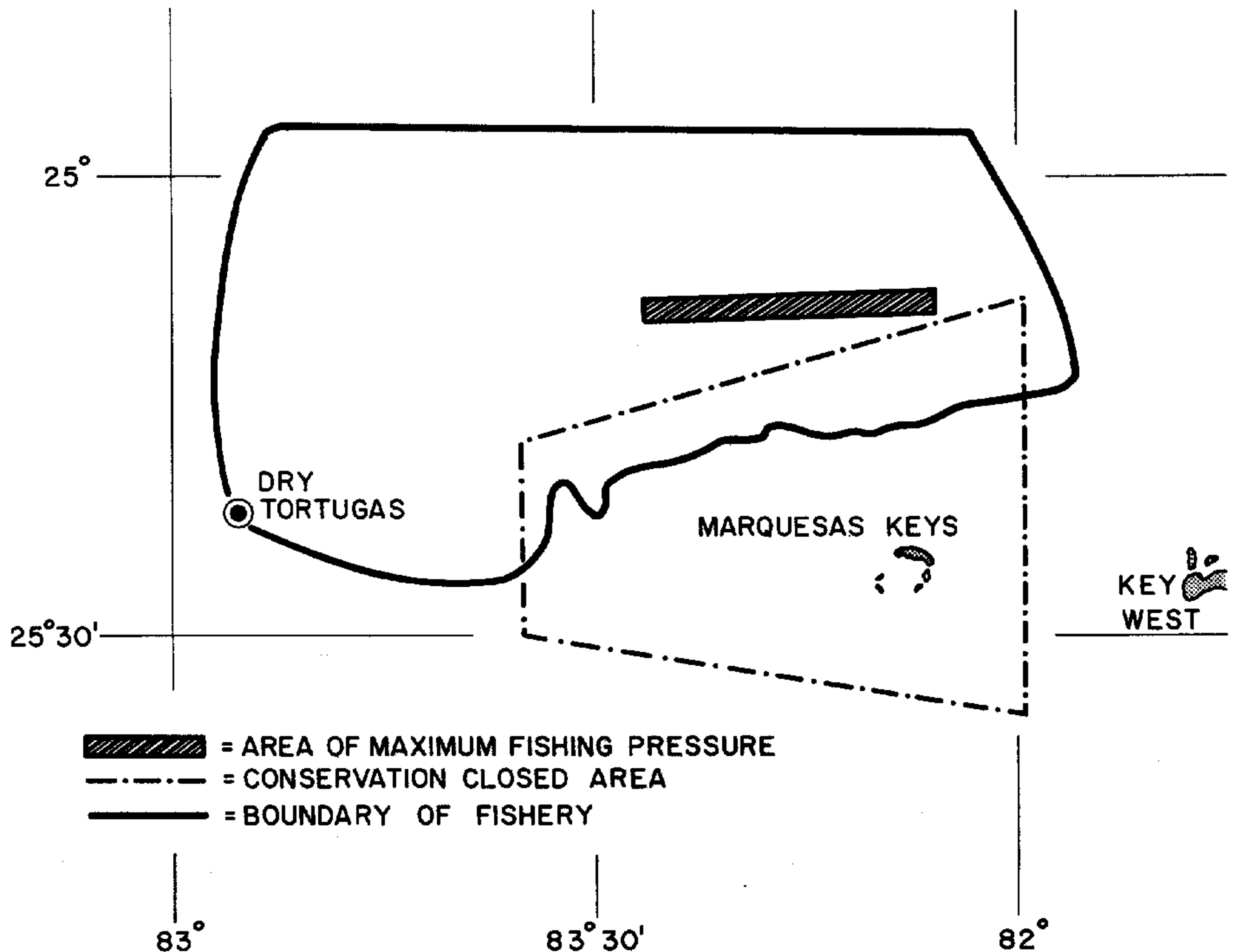


(N) = MEAN COUNT/POUND (HEADS-OFF)

CIRCLE DIAMETER INDICATES AVERAGE LENGTH IN 10 MILE SQUARE

SAMPLES FROM 73 DRAGS; OCTOBER, 1957 - FEBRUARY, 1958

Size-depth relation of female pink shrimp in 73 drags on Tortugas grounds.



Showing Tortugas fishing area and portion of heaviest fishing concentration.

An additional observation of interest related to the Tortugas fishery is the new shrimping area in Hawk Channel at Bahia Honda Key, South of Marathon, Florida. Good catches were made in this new area in February when weather conditions prevented shrimping on the major Tortugas grounds. More exploration may expand this relatively new Tortugas fishery even more.

SHRIMP MARKING

T. J. Costello, Jr.

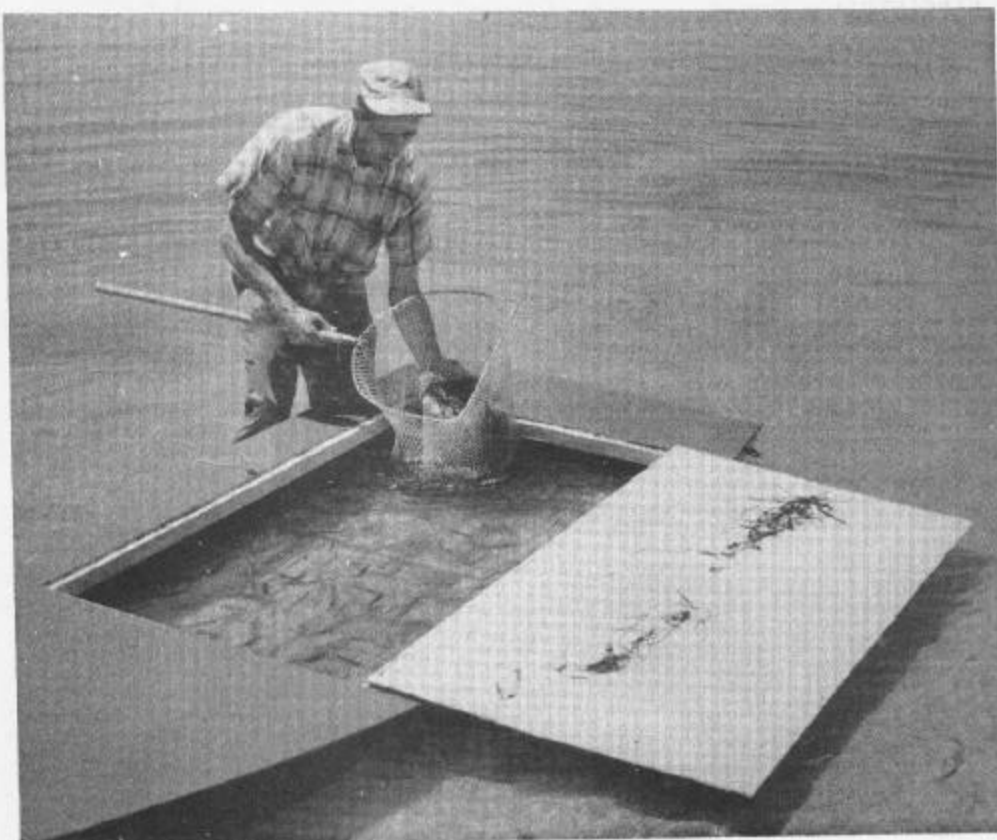
Charles Dawson developed a new and promising method for using biological dyes to mark penaeid shrimp working under a Saltonstall-Kennedy contract with the Fish and Wildlife Service. The initial work in determining methods for field application consisted of laboratory studies in Galveston which repeated some of the original studies and brought to light, among other things, the importance of using a standard artificial sea water base as the stain solvent in order to achieve consistent results and keep staining mortality low.

The second phase consisted of a small staining experiment conducted in Lufkin Bayou near Galveston. Shrimp held in "live cars" were fed mullet containing Typan Red. Injections of Fast green and Typan blue were given to two thousand brown and white shrimp. This brief study added materially to our knowledge of what problems would be encountered in a large field marking program. A decision was reached to carry on this marking program in Florida applying the method to Florida pink shrimp.

At the Marine Laboratory of the University of Miami, repetition of some of the staining work showed the pinks, *P. duorarum*, somewhat better subjects for the dyes than either *P. setiferus* or *P. aztecus*. Methods were developed for separating large groups of pink shrimp into size groups and toxicity thresholds were determined for each size group.

With technical details complete, it was decided to stain the first large group of pink shrimp in Biscayne Bay and follow this with a larger program in the Whitewater Bay area of Everglades National Park. This plan required that a fully portable field station be designed and built, a station which could be carried from place to place and set up wherever shrimp, in sizeable quantities, were to be marked. With equipment assembled, shrimp for marking were obtained from regular bait shrimp fishermen working rigid frame trawls in Biscayne Bay.

Full scale marking and releases began April 24. By the end of May 20,000 shrimp stained with Fast green had been released in Biscayne Bay. These shrimp, clearly marked, immediately began to show up in the regular bait shrimp catch. By May 10, several reports were received of marked shrimp noted by shrimp fishermen. In the first 5 weeks after releases began 26 were actually recovered in



2,000 stained shrimp in live car ready to be towed to release site.

addition to many others reported. This attests to the usefulness of the new method. With sufficient coverage of a fishery marking with biological dyes should be a very useful new method of obtaining information on pink shrimp. Naturally, we hope some of the shrimp marked in Biscayne Bay will be recovered elsewhere furnishing a clue to where the sizeable populations which pour out of this bay spend the mature stages of their life.

The efficiency of recovery depends on the handling of the shrimp after capture. Thus a bait fisherman noted no stained shrimp while making a catch of 12,000 but the next morning, while counting them out individually for sale as bait, he found 7 that had been stained. The

shrimp were all clearly marked and easily recognized when they were handled individually as they were in this instance. Usually bait shrimp are not counted individually, but are estimated on the basis of weight per hundred. When they are in short supply, as they were recently, they are counted individually and then the stain shrimp are easily noticed. This points up the necessity for thoroughly covering the commercial fishery as we extend marking to the Tortugas populations.



Marking pink shrimp by injection of colored dyes.

For a period of 3 to 5 days following injection of the stain the shrimp's tissues are all stained. The dye then concentrates in the bronchiae and the stain remains clearly visible in this area for several months. An analysis of the stained shrimp which have been recovered by the bait fishery shows that all but one of the recovered individuals are probably shrimp which have been released at least 3 days. That is, only one shrimp with stain in other tissues than the bronchiae has been taken. This is probably because the stained shrimp, in some distress

from the injection, have more of a tendency to "bury up", that is sink into the sand, than shrimp which have been stained for a few days. This has been noted in the controls. Staining mortality, based on observations at the time of staining and on controls held several days, has been surprisingly low--about 5 per cent to date. From recoveries to date, there appears to be a general movement northward in Biscayne Bay toward Bear Cut which opens into the Atlantic. One stained shrimp had traveled approximately 9-1/2 miles from the release area.

MORPHOLOGY OF SHRIMP

Joseph H. Young

Tulane University

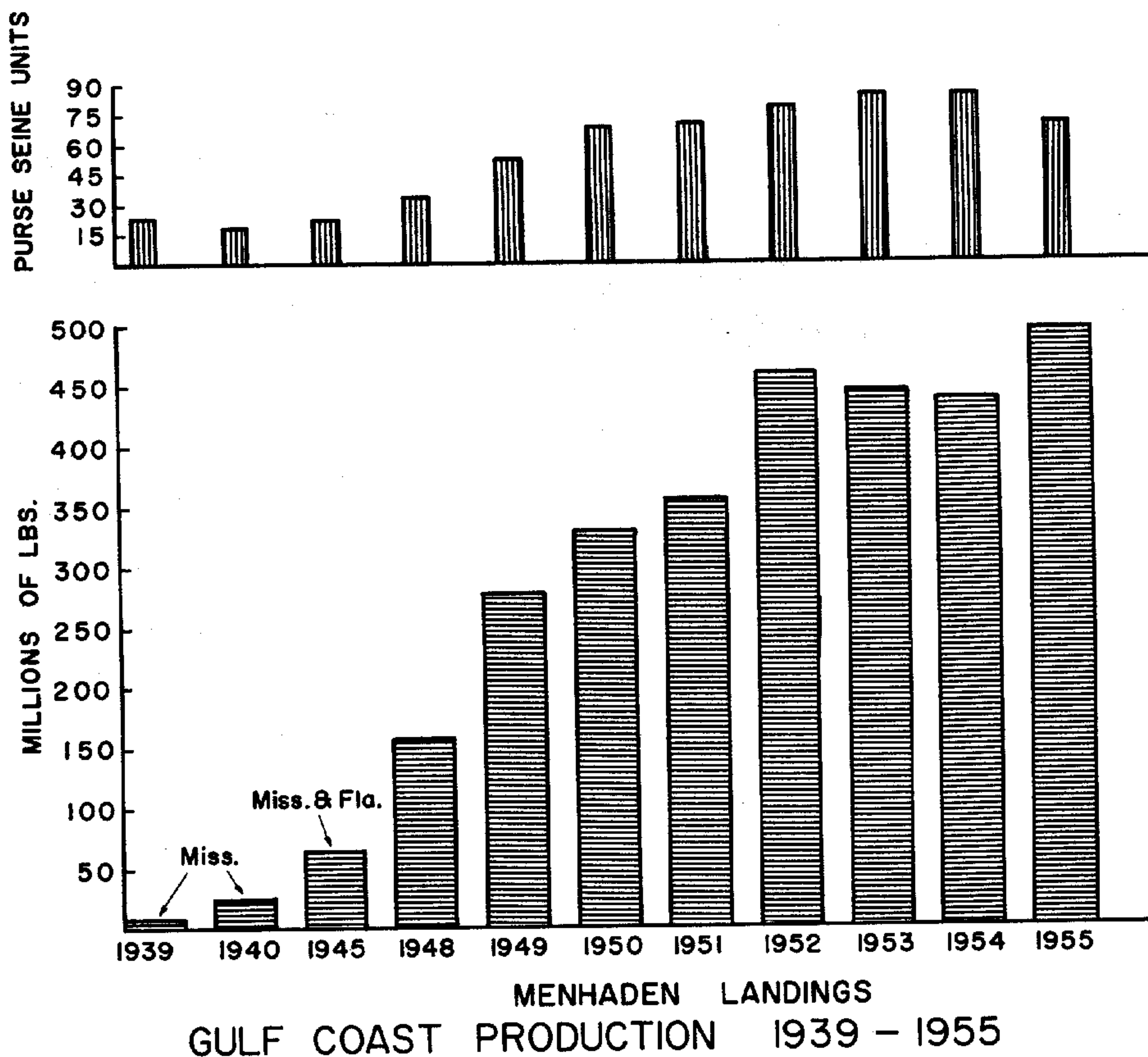
(Contract No. 14-19-008-9348)

An atlas of the white shrimp, Penaeus setiferus was completed and is now being printed in the Fishery Bulletin of the Fish and Wildlife Service. An atlas of the brown and pink shrimps, P. aztecus and P. duorarum is being completed.

AGE AND GROWTH OF MENHADEN

Edgar L. Arnold, Jr., Project Leader

During the past twenty years annual landing of menhaden by the commercial fleet from Gulf of Mexico waters have increased from 10 million to over 500 million pounds. The accompanying graph, based on catch statistics compiled by the Statistical Section, Bureau of Commercial Fisheries, illustrates the growth of the industry throughout most of this period.

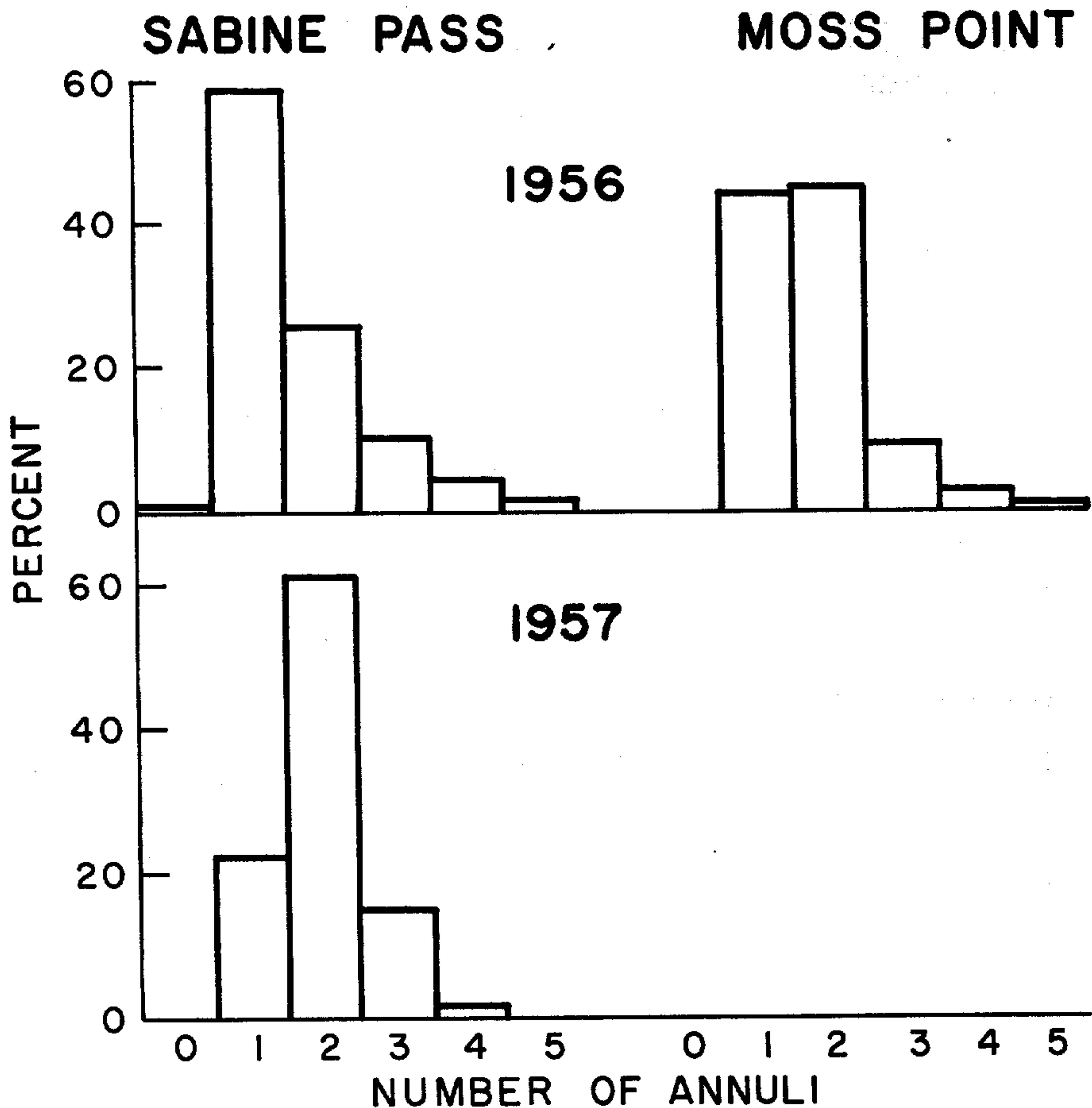


Gulf Coast Menhaden production

The fishery depends almost exclusively on one species of menhaden, Brevoortia patronus, the Gulf of Mexico counterpart of Brevoortia tyrannus of the Atlantic seaboard. Another species, B. gunteri, appears sporadically in commercial catches made west of the Mississippi River, but its contribution to the fishery is probably less than 0.5 percent.

To determine whether or not the population of Gulf menhaden could maintain itself indefinitely under the increased fishing pressure, the Gulf Fishery Investigations began a study of the fishery in the fall of 1955. Knowledge of the year class composition of the commercial catch from year to year provides a measure of the impact of the fishery upon the species, but more importantly, provides a measure of annual spawning success, and the resulting changes in sizes and numbers of the individual fish. Accordingly, we have been investigating the legibility of scale annuli as a means of obtaining individual ages and consequently the desired estimate of the year class composition of the population.

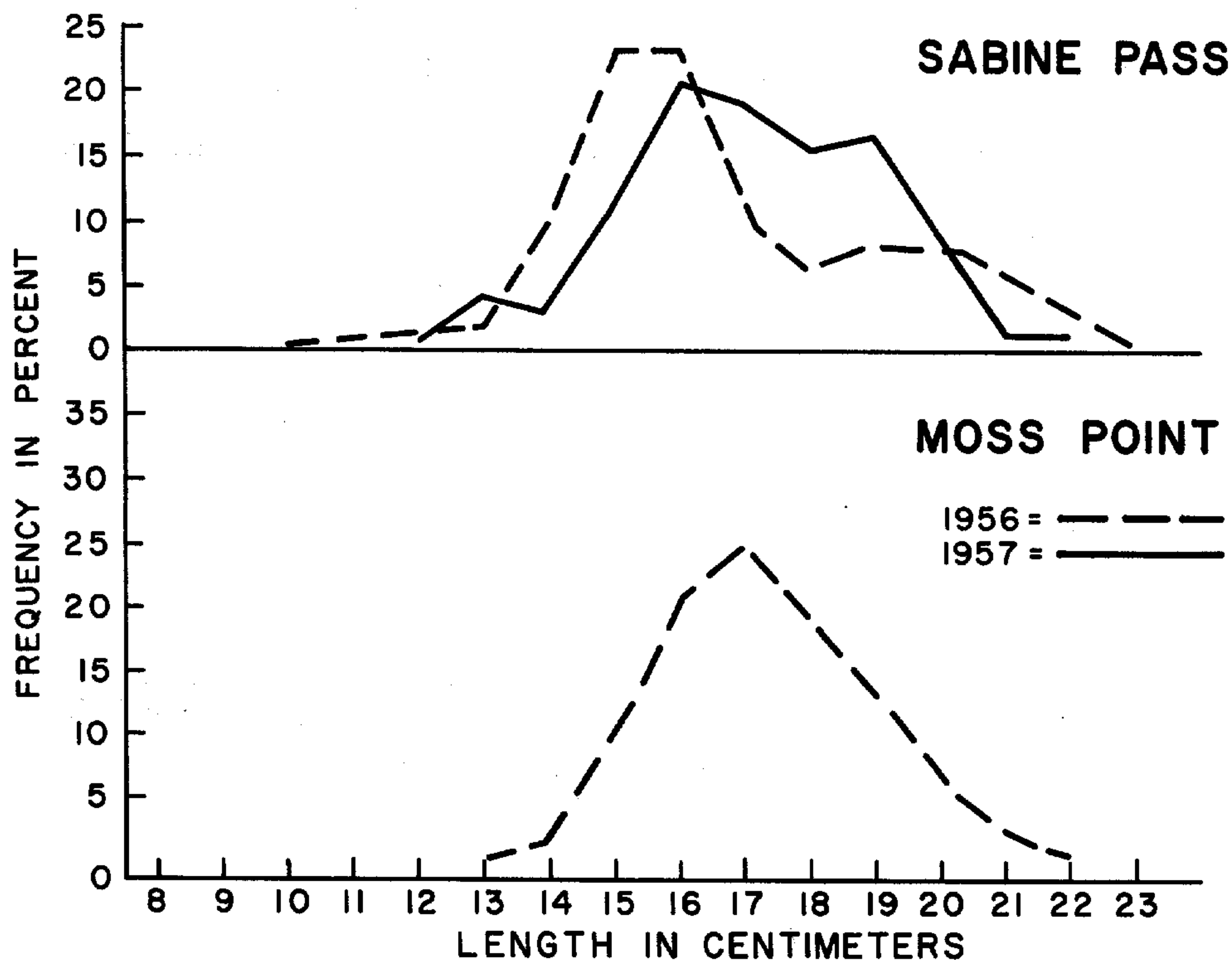
Periodic samples of the commercial catch for the 1956 and 1957 seasons were obtained from landings made at Sabine Pass, Texas, and Moss Point, Mississippi. Treatment of samples included the taking of lengths, weights, state of gonad development, and scales for age determination. Use of the impression method for preparing scales was explored, but found unsatisfactory. Actual scales, mounted between two glass slides, were used for reading by means of a micro-projector with a magnification of 40 diameters. Some difficulties were encountered in determining the annuli, with approximately 10 percent of the scales proving to be illegible. The figure shows the age composition, by percent, of samples obtained from the commercial catch for 1956 and 1957 from Sabine Pass, and 1956 from Moss Point. Readings of the 1957 Moss Point samples have been completed only through the 8th of August, and are not included in this report. Time did not permit a check of many of the readings by a second reader, and results, therefore, must be accepted with reservation.



Age distribution of menhaden for 1956 and 1957.

It appears that one and two-year old fish comprise the bulk of the catch, with three-year olds making up approximately 10 percent in both areas. The apparent anomaly of the percent composition of one and two-year olds for Moss Point in 1956 is inexplicable at present, but may be resolved when readings are checked, when 1957 readings are completed, and catch data analysed. Length frequency data (see accompanying

figure) shows that over 85 percent of the fish in the commercial catch were between 14 cm. and 19 cm. fork length, with a total range between 7.5 cm (one fish) and 23 cm.



Length distribution of menhaden for 1956 and 1957.

Similar sampling and treatment of samples will be continued for the current season. Marginal increments (proportionate distances between annuli from the outer annulus to the scale periphery) will be analyzed for determination of growth rates. The investigation will be expanded, in 1958, to utilize facilities of the Exploratory Fishing and Gear Development laboratory at Pascagoula, and will include distribution and life history studies of menhaden and other industrial fish throughout the Gulf of Mexico. The possibility of tagging also will be explored to obtain data on movements and growth.

Observations and collections of marine forms in the waters adjacent to Galveston Island, principally in East Beach Lagoon, have been carried on periodically since 1953. Analysis of data thus obtained reveals that spawning of B. patronus extends over a considerable period, agreeing closely with studies by previous workers. Larvae and post-larvae first appear in mid November, and still are present in June, mixed with schools of juveniles from the early spawning. No specimens to date have been identified as B. gunteri, although large adults (22-28 cm) in spawning condition were taken from time to time along the Gulf beaches, and several specimens (29-30 cm) were obtained from the commercial catch at Sabine Pass. It should be noted that while B. gunteri has been considered as a brackish-water species, we have found them only on the beaches or slightly off-shore, never in the bays or estuaries.

While by no means a prediction for next year's fishing, juvenile menhaden are present in local waters in concentrations far exceeding those of previous years, and according to information from the Gulf Coast Research Laboratory at Ocean Springs, Mississippi, the same holds true for that area.

MENHADEN DEVELOPMENT

Royal D. Suttkus

Tulane University

(Contract No. 14-19-008-9344)

This contract was initiated on May 16, 1957 for the purpose of describing and illustrating developmental stages of larval and young Gulf of Mexico menhaden.

Until recent times only two species of menhaden were known to occur in the Gulf of Mexico. While collecting samples along the west coast of peninsular Florida during November, 1957, the investigator obtained menhaden specimens from near Placida, Florida. These specimens were identified as Brevoortia smithi. Visits to several museums and a considerable amount of correspondence revealed the presence of additional B. smithi specimens that had been overlooked by previous workers, namely Samuel F. Hildebrand. These preliminary observations were followed-up by a presentation of a paper ("Distribution of Menhaden in the Gulf of Mexico") at the 23rd North American Wildlife Conference.

Half-tone illustrations have been completed for the following species and sizes (standard length in mm.).

Brevoortia patronus: 19.2, 20.0, 21.3, 22.3, 23.7, 24.7, 25.7, 26.8, 28.2, and 31.0. Two of these illustrations are included in this report.

Brevoortia gunteri: 22.5, 25.2 and 29.9

Brevoortia smithi: 22.4 and 25.2

Harengula pensacolae: 20.7

Anchoa mitchilli: 21.5

Dorosoma petenense: 20.9

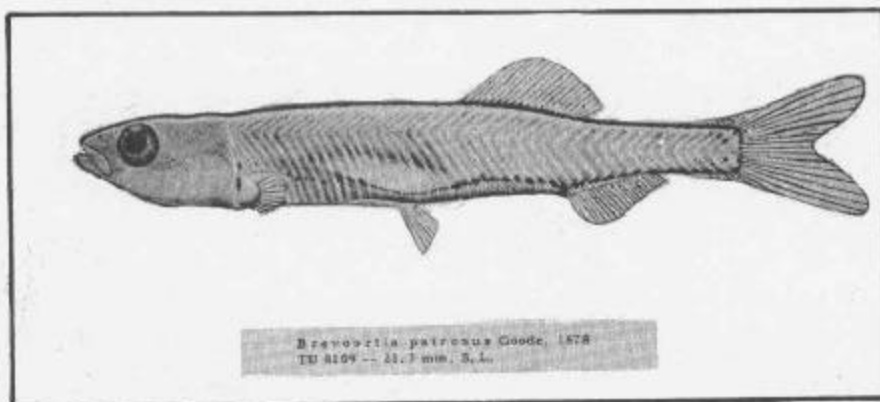
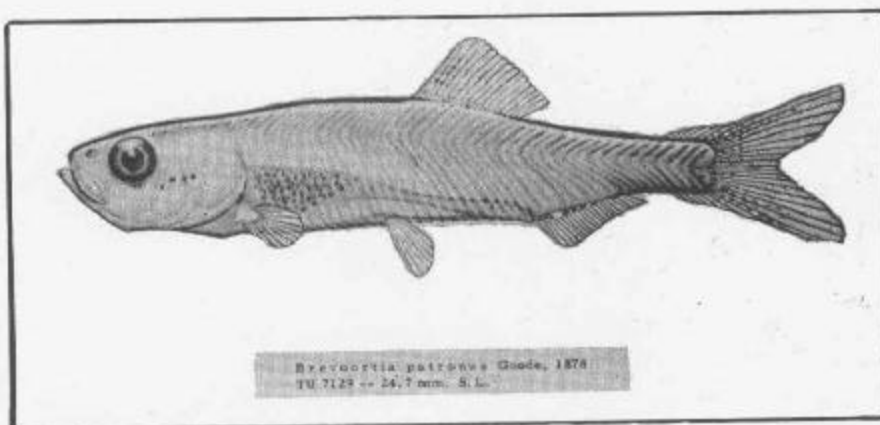
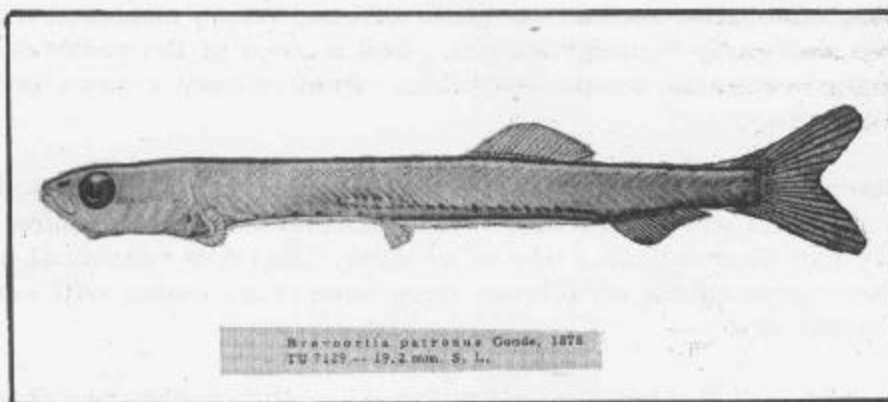
Considerable work has been done with regards to determining differentiating criteria for the three species of menhaden. The analysis of materials recently obtained from Florida, Mississippi and Texas coasts should enable me to establish the validity of the criteria mentioned above.

The pigmentation patterns and differential development of proportions seem to be the best features for differentiation of certain of younger stages of the three species. The number of ventral scutes,

scales, and opercular striae are good differentiating characters for late postlarvae and early "young" stages. Soft x-rays of the postlarvae should make vertebral counts available. Preliminary x-rays have been very satisfactory.

Samples of nearly all the related clupeids and engraulids that occur in the area where postlarvae of menhaden occur have been studied externally and internally (by use of x-rays). Besides vertebral counts a few other osteological structures have been found which will serve for identification.

I believe that when the full potential of the menhaden resources in the eastern Gulf of Mexico is realized it will have a decided impact upon the menhaden fishery.



Post-larval and juvenile menhaden

MENHADEN POPULATIONS

Gordon Gunter

Gulf Coast Research Laboratory

(Contract No. 14-19-008-9335)

Introduction

The menhaden work here was one year old on April 30, 1958. The biologists spent the first few months getting the boat ready, buying equipment, making trips with menhaden boats and surveying the literature. The literature survey resulted in a bibliography of the menhaden, now being prepared for publication in collaboration with Mr. John W. Reintjes, and a review of the catch statistics of the Gulf fishery and the biology of the Atlantic and Gulf menhaden. During May, June and September 1957, the biologists made five trips with menhaden boats. They also spent some time talking to plant operators and menhaden fishermen. The information gained was turned in as a separate report.

During June, July and August 1957, Mr. Bennie Rohr collected length measurements and scale samples of fish from the commercial catch brought into Moss Point (Pascagoula) and transmitted them to the Galveston office for use in the aging study.

Plankton Collections

The plankton tows were started in November. Beginning plankton hauls were all picked over for fish eggs and larvae. Up to February 22, sixty-eight hauls were examined and found lacking in eggs and larvae of any kind. During the same period, seventeen tows yielded menhaden larvae and postlarvae. The clupeid larvae were picked out and sent to Dr. R. D. Suttkus. All of the remainder of the samples were discarded. We kept part of a sample, thought to be small menhaden larvae; they are about 9 mm. long. In late February, we stopped examining the tows and merely shipped half of each sample. The first menhaden larvae were taken on December 16 at the mouth of Biloxi Bay. The smaller larvae were taken in the Gulf and in general they seem to become larger as the more estuarine waters are approached. The numbers of larvae seemed to decrease in April. Large numbers of fish eggs were taken in April. A few tows were made at depths of about two fathoms with the half-meter net. They yielded no larvae, and all remarks here refer essentially to surface tows.

In summary, we have taken 131 plankton tows in search for menhaden eggs and larvae in the waters of Texas, Mississippi, Louisiana and Florida. Sixty-seven of these were known not to contain larvae. Seventeen were known to contain larvae or postlarvae ranging from about 9-40 mm. Fifty-seven samples were turned in to Doctor Suttkus unexamined.

Beach Seine Collections

A program of beach seining with fine mesh nets was initiated in March. During that month three hauls were made on the Gulf beach west of Sabine Pass and three were made along the shores of Sabine Lake. Fifteen hauls were also made at a series of stations in the Mississippi Sound and Biloxi Bay. In April twelve stations were made again in the same area, and up to the time of this report three were made in May. A summary of this information is as follows:

Total number of menhaden recorded - 21,075
Total number of hauls - 37
Range of catch per haul - 0-5550
Average catch per haul - 569.6
Range of size taken - total length - 21-46 (one specimen
total length 84 mm. was taken)
Range of salinity - less than 1.84 - 30.77
Total fishes recorded - 32,052, with 32 species included.

In general the little menhaden ranged from 23-33 mm. in total length in early March in the Mississippi Sound area. In early May they were 28-41 mm. long. The size changes do not show so well in limit measurements and only length-frequency curves will show the complete picture. Small fish were found on the outside beach of Horn Island in early March, but they all moved to inside waters in April and at present are distributed along the shores of the whole Mississippi Sound area and its tributaries. The small fish seem to seek low salinity areas or shore areas; they seem to be relatively indifferent to salinity and were found over a wide salinity range.

Gill Net Sampling

Work with gill nets as a means of sampling the menhaden population was begun in September. Fifty fathoms of two inch stretch mesh nylon webbing was hung to fish ten feet deep. When set from an outboard skiff around menhaden, schooled at the surface in shallow water, this net took fish satisfactorily. Several attempts to locate fish in deeper water for further testing this method of fishing failed. Stop net fishing took many menhaden when they were located where this method could be used. Only

a few menhaden were taken by setting from the beach and hauling out. Night fishing, with the net anchored in deep water and fishing from the surface down proved successful. Boat anchors attached to the lead line with sufficient cable held the net in position. In the open Gulf, regular drift fishing procedure was followed.

Fifty fathoms of 2-3/4 inch stretch mesh net fifteen feet deep was hung in December. Early in January the two nets were sewed together. As was expected, the gill net proved to be distinctly size selective. With the exception of a group of 120 to 159 mm. fish taken in the 2-3/4 inch mesh net, there is only a slight overlap in the length frequency curves. An examination of the raw data reveals that these were all taken in one set when the water temperature was 11.4° C. When the net was picked up, many of these small fish were seen to drop back into the water. None was properly gilled. The fish seemed to be inactive when taken aboard, although the net had been out only about two hours. It seems likely that the low temperature of the water had partly stunned these fish and they simply stopped against the net when they hit it.

In the combined data over 90 per cent of the fish taken fall into two modal groups covering a range of about 55 mm. each. This indicates that a single mesh size may be expected to take fish with a total length range of about 55 mm. It follows that gill net size selection of menhaden may be largely eliminated by building a net of equal lengths of mesh sizes at 1/2 inch stretch mesh intervals. Fish taken in the large mesh net were larger than the fish taken by the commercial fishermen. These large fish were what we were after. However, no spawning schools or ripe fish were taken by this method, and in this respect the operation was a failure. Quite large fish were taken in inside waters until about mid-January. After that they diminished sharply in numbers and apparently moved out into deeper waters. These fish were all used or stored for use in the racial studies.

Otter Trawl Sampling

A review of data taken in a survey of Mississippi Sound fauna using a flat otter trawl on four stations transecting the Sound and extending into Biloxi Bay revealed that only 66 menhaden were taken in 58 hauls extending from December 1956 through November 1957. Thirty of these specimens were taken in one haul in September. All other specimens were taken in the months of April, May, October, and November. Total length of these specimens showed a range of 99 to 212 mm. All stations were run in daylight hours. In order to check the impression that a flat otter trawl would be an unsatisfactory net for sampling the menhaden population, approximately 150 additional hauls

were made under varying conditions and extending to 15 fathoms in the Gulf. The number of menhaden taken was insignificant. On one occasion in June, the "Hermes", with Dr. H. J. Bennet and a group of students aboard, made a trawl haul around one of the menhaden boats while fish were being pumped. No Brevoortia were taken in the trawl although many were known to be in the vicinity. Numbers of the Menhaden Investigation staff were taking specimens from the commercial haul and observed the trawl operation, which was carried out at their request.

In April, a four seam balloon trawl with wings ten feet deep was put into service. Present indications are that this net may become an important part of our sampling technique. We feel that the balloon type trawl is the answer to capturing samples of spawning or pre-spawning schools. On March 7, 1958, the "Oregon" took approximately 1,200 pounds of menhaden in a condition which was within two days of spawning, according to Mr. Harvey R. Bullis. Their gonads were very large and obviously very ripe. A pre-spawning school of this type should be fully aggregated and separated into the various stocks, if such stocks exist. All observations and information indicate that such schools are found offshore and below the surface in the winter and will have to be captured by trawls.

Development of Gonads

All fish examined through the end of August 1957 were in the resting stage, with the gonads small and firm in texture. Early in September some specimens were observed in early developmental stages. Gonads were noticeably larger and microscopic examination revealed that ovaries were crowded with developing eggs. All fish did not begin development at the same time. By mid-November the gonads of many specimens were well developed. Eggs in all ovaries examined were of two or more sizes. This condition continued through the winter and spring, into the month of April.

On April 4 a sample of 35 specimens ranging from 165 to 254 mm. in total length, included spent and full Brevoortia patronus. Seven fish, all over 200 mm. total length, were not spent. In some schools of menhaden gonadal development shows extreme variation. Gonads of fish of the same size and from the same school have been found nearly fully developed and at resting stage. The most extreme example of this is a sample from off Mississippi Sound in which 23 per cent of the fish showed no signs of development, whereas 77 per cent were in various stages of development. The fish were relatively small with a mean fork length of 139.09, but at the same time the

largest portion of them were developed to the point where they would probably spawn in the spring of 1958. The undeveloped gonads could possibly be caused by a parasite. In the sample from off Pass a la Loutre, taken by the "Oregon" in March 1958, every fish had fully developed gonads which appeared to be very close to spawning.

Developing gonads were observed in menhaden from about 140 mm. total length to the largest taken. The color of the fresh ovaries varied considerably. Whether this color difference could be used as an indicator of maturity has not been determined. The presence of fully developed gonads in fish found in estuarine areas during the winter, and the absence of spent fish, indicates that at least a part of the population remains in low salinity areas until the reproductive organs are nearly ripe. They then move offshore to higher salinities where spawning takes place. Some spent fish remain offshore until the spring and summer movement of schooling menhaden. Asymmetry of the gonads is noticeable in many of the specimens examined. The left gonad is consistently smaller than the right, where asymmetry is marked.

The length of the spawning period may imply that there could be stocks of menhaden separated in time. Work thus far has been predicated primarily on the basis of stocks separated in space. Future work should take into consideration the possibility of early and late spawning groups.

Meristic Examinations

Selection of the Sample Areas

The three sample areas selected were Apalachicola Bay, Florida, the Mississippi Sound area, and Sabine Pass, Texas. Each of these areas is affected by different river systems and also separated geographically by many miles. Also, these are the areas which contain enough menhaden to support commercial fishing. A fourth sample was taken off Pass a la Loutre by the Fish and Wildlife Service vessel "Oregon". The fish appeared to be nearer to spawning than any other sample. Menhaden depend upon estuarine areas for a complete life cycle, indicated by the fact that the young swim into low salinity bays, bayous, etc., for the early part of their lives. It seems that large river systems causing estuaries would support separate stocks, if such exist. It is possible that menhaden move to the areas affected by these rivers during times of spawning so that the young would have easy access to the estuaries.

Methods of Enumeration and Proportional Measurements

Oblique rows of scales were counted, beginning at the most posterior edge of the opercle and ending at the base of the caudal fin. The base of the caudal was considered to be a line between where the dorsal and ventral portions of the caudal entered the peduncle. By use of india ink to accentuate the scales these can be made exact. Oblique series of scales were not counted on the Pass a la Loutre sample because nearly all fish were damaged.

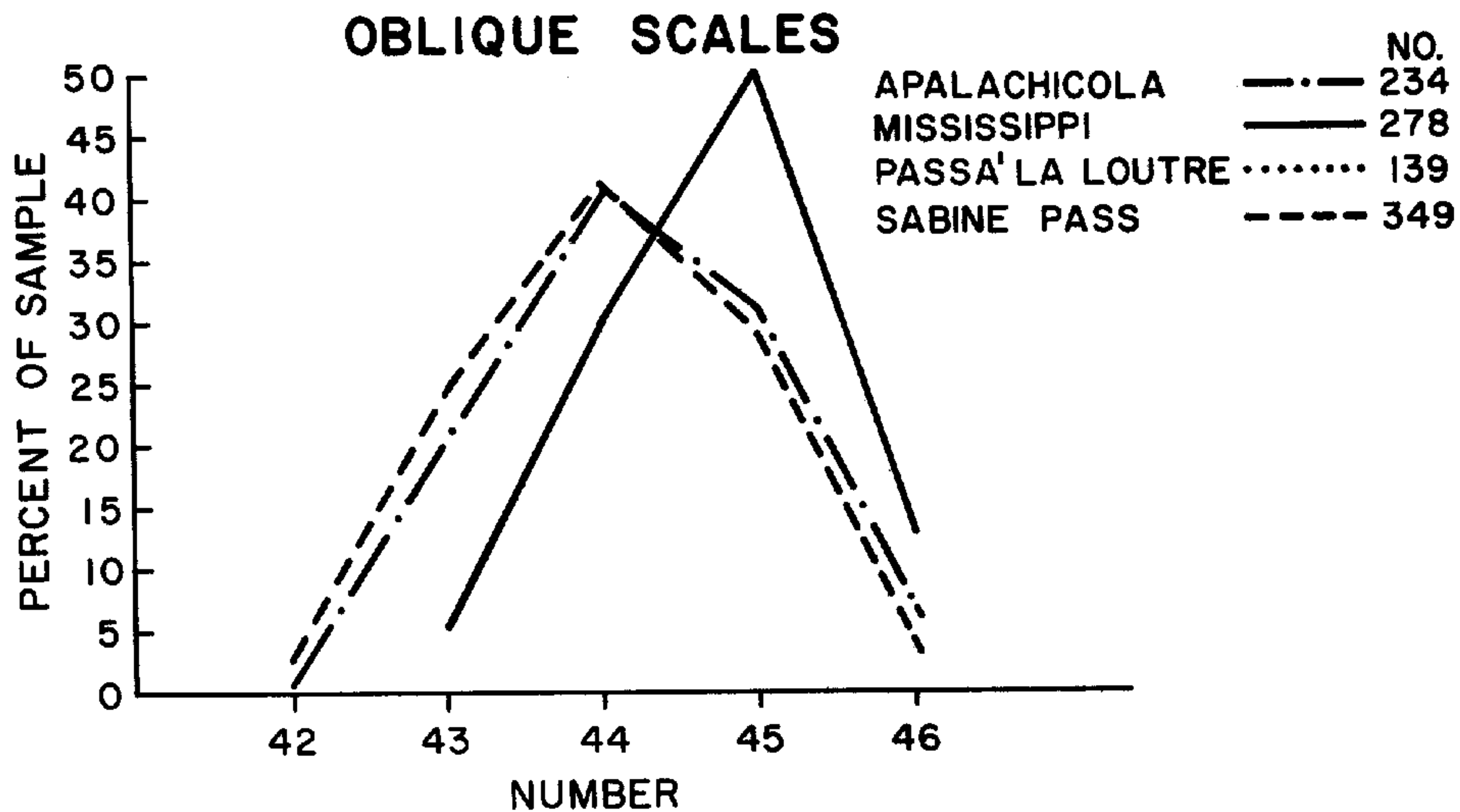
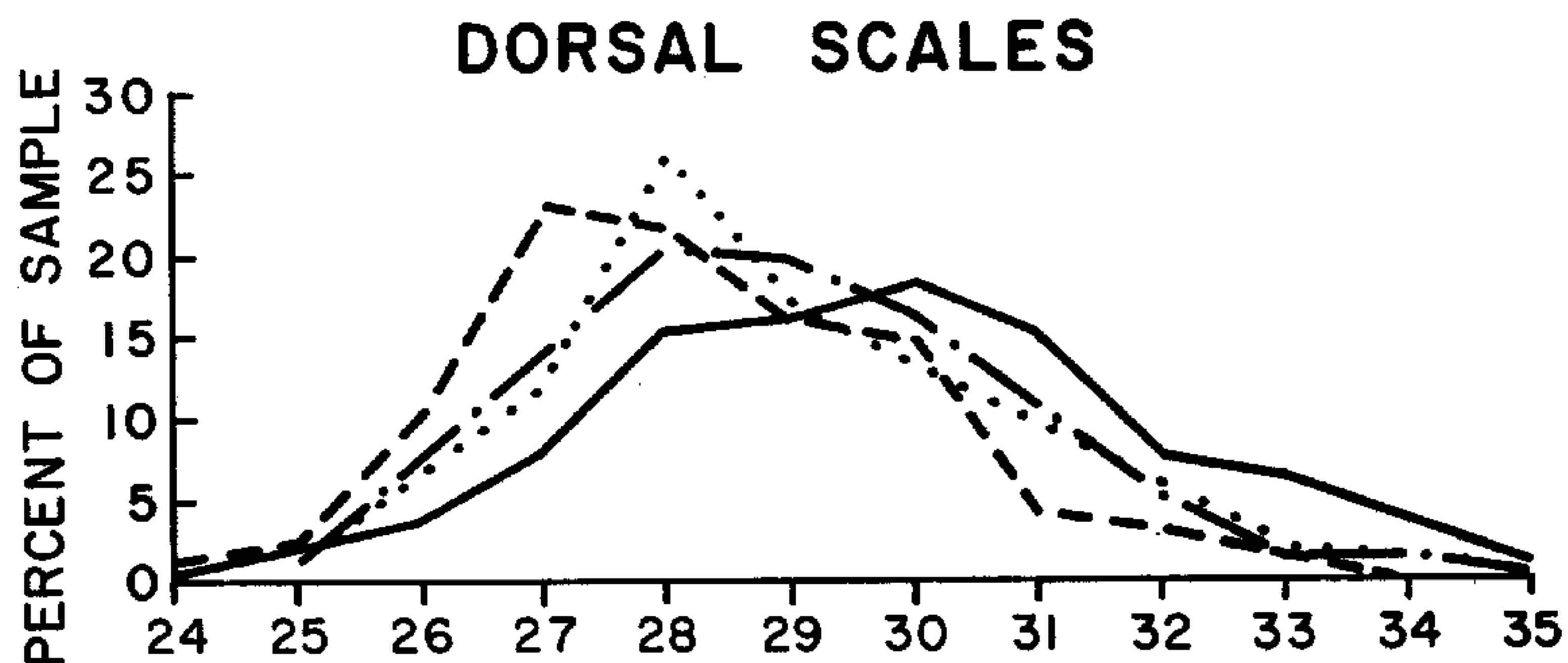
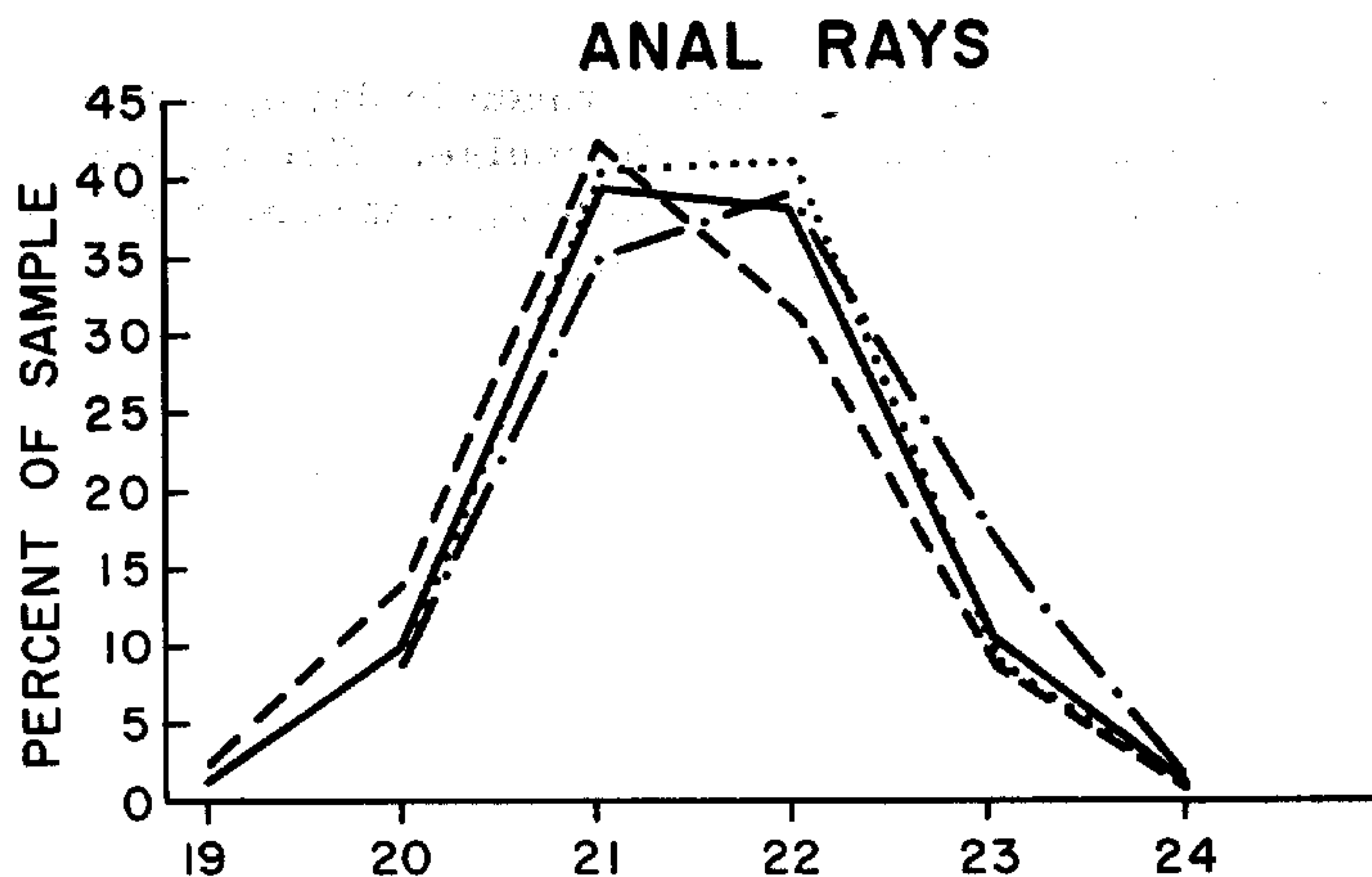
The number of modified scales on the back in front of the dorsal includes only those with a free lateral edge. This characteristic had a wider range than any of the others, and adult numbers were shown on fish as small as 100 mm. fork length. Smaller fish were not examined meristically. Fork length was measured from the tip of the closed mouth to the fork of the caudal. Fork length was used in preference to total length because the tips of the caudal are easily broken. Standard length was not used because it is a slower method and not as exact.

Some differences were noticed which could not be measured or counted. The ventral scutes of fish from Apalachicola Bay were much more prominent than on fish from the other sample areas. The offshore sample obtained by the "Oregon" were by far the most advanced in reference to gonadal development. The gonads could not have gotten much larger since they filled about nine-tenths of the abdominal cavity and the eggs appeared to be fully mature. Sperm and eggs were placed together in a dish but no fertilization was observed. The dark spots on the sides of the fish were much more uniform in size, no deformities were noticed, and the overall physical condition of the fish appeared to be excellent.

Results

The figures are frequency plots of the various counts of the four samples. All of these fish were caught in our operations, except for the Pass a la Loutre sample taken by the "Oregon". These fish were taken on March 7, 1958 in a mid-water trawl at a depth of about 7 fathoms in 14-16 fathoms of water. Since they were a spawning school, they were plotted separately. In general fish from this school seemed to be not quite so variable as the others, especially in vertebral count and ventral scutes.

The figures given are for characters which show the greatest differences, oblique scales, modified dorsal scales and anal rays. The other characters show little variation from place to place.



Meristic characters of menhaden in four localities from Apalachicola, Florida, to Sabine Pass, Texas.

These frequencies are the only analyses made to date, because we want more data before applying statistical formulae. For the present there are no clear indications, one way or the other, that separate stocks of Brevoortia patronus exist in the northern Gulf.

ZOOPLANKTON

Abraham Fleminger, Project Leader

In familiar boreal waters such as support the herring-mackerel and haddock-cod fisheries of North America and Europe, the role of zooplankton is well established. Briefly, zooplankton lies in the very center of the dynamic cycle of marine production, mediating between the potential of a water mass and its production of finny fish. It, therefore, follows that details of zooplankton composition, gross seasonal abundance, rate of turnover, and geographical distribution are fundamental to the question of fishery management. According to the recent literature this information can also provide a means for estimating or predicting matters of such immediate interest as the quantity and quality of pelagic fisheries in a given region, the degree of success that can be anticipated for a particular year's spawn, that type of fishing gear that will be best for pelagic fishing at any given time and place, as well as migratory routes and feeding area of pelagic fishes. Appreciation of pelagic community dynamics, especially as regards maintenance and growth, can be aided materially by quantitative and qualitative information of plankton community structure, its environmental needs, and its means of distribution. Incidental to this, study of animal plankton has further utility in its applicability to hydrographic studies. Besides its value in serving as a check on water movement determined by calculations using physical and chemical measurements, under certain circumstances it provides the only source of measurement of hydrographic change available at present.

Prior to the inception of Gulf Fishery Investigations virtually nothing was known of the animal plankton populations in the Gulf of Mexico. Initially, then, the composition of this fauna, its distribution, and basic biological data on numerically important species inhabiting the surface layers were the first questions to be answered. Complicating these needs was the fact, that the basic pattern of water movement both within the Gulf and between the Gulf and adjacent seas are in dispute: in general a local plankton fauna will reflect the major water masses which drift through the region. Determination of the fauna and its distribution within the Gulf makes possible comparison with faunas of adjacent seas which could be helpful in settling controversies.

This comparative study has the additional value of providing the means for initiating new consideration of the influence of temperature change on pelagic waters. As is well known, the more northern latitudes have been experiencing a gradually warming climatic trend that has had

a conspicuous effect on the distribution and abundance of bottom invertebrates and ground fish. It is of interest to see to what extent qualitative changes in the zooplankton occur in response to geographical temperature gradients. Conceivably plankton animals could provide rapid and relatively inexpensive means of assaying these changes as they influence local hydrographic conditions. In European waters, for example, the origin and relative fertility of water masses entering and mixing in the North Sea are being studied through the use of plankton animals. The evidence accumulating from these studies indicates that one will be able to predict with reasonable success the outcome of a particular year class of fish at about the time of spawning.

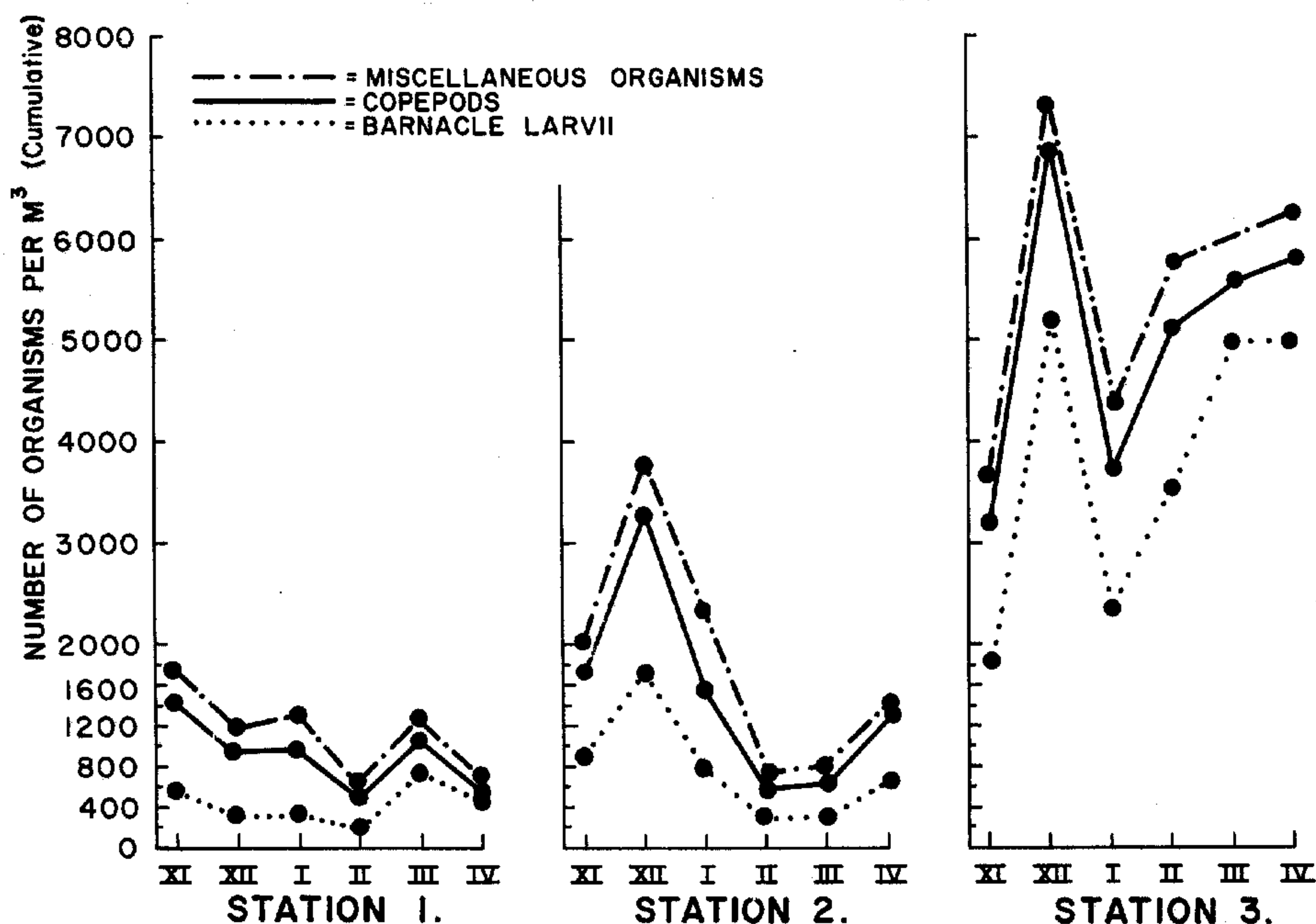
Zooplankton of inshore waters, especially in semi-enclosed areas behind barrier islands and in broad embayments with restricted mouths, is also of more than academic concern. These areas are the most productive shrimp nursery grounds, and the interrelationship between animal plankton and shrimp, especially as regards growth of the latter, is an unknown one.

In establishing a basis for evaluating the zooplankton in the Gulf of Mexico study commenced with the calanoid copepods since they comprise the numerically dominant group. Investigation of offshore waters beyond the 10 fathom contour has been accomplished by means of the plankton collections taken by the M/V ALASKA (1951-1953). Collections from inshore open coastal waters are being obtained whenever and from wherever possible. Fauna of the northern Gulf coast is now fairly well established and there is hope that, with the cooperation of the U. S. Coast Guard, a more adequate survey can be made of the Gulf of Campeche and Yucatan shelf waters. Regarding the more protected back waters behind barrier islands and within the broad shallow bays some qualitative data has been obtained from several localities over the northern coast and preliminary quantitative study is now in progress on the East Beach Lagoon of Galveston Island in conjunction with the Ore Dike Program.

The calanoid fauna recorded thus far in surface and subsurface waters totals about 135 species representing 53 genera and 23 families. Regarding only species about which an estimate can be made of its usual environmental range, the fauna is comprised of the following: 34 surface species, 75 subsurface species, 28 inshore species and 79 offshore species. Of these, about 16 surface species, 18 subsurface species, 11 inshore species and 23 offshore species occur with sufficient frequency to require future detailed study in respect to their importance as food for the surface and mid-water pelagic fishes in the Gulf region. Biogeographically, the offshore copepods are tropical-subtropical in character. Evidence of seasonal variation is apparent only in the temperate, northern inshore

waters between the northern halves of the Florida and Texas coasts. The calanoid copepods in this region are typically warm-temperate (Carolinian), their range in the Gulf coinciding with the range of the white and brown shrimping grounds. The pink shrimping grounds occur in waters with a definite tropical character regarding both temperature and plankton fauna. Several interesting changes in the calanoid fauna of the Gulf shelf furnish excellent clues as to the tropical and temperate nature of the different regions. As in the penaeids replacement of shelf species involves several cases in which the tropical species are in the same genus as the temperate species.

Before taking up the question of what can be inferred from the comparison between the faunas of the Gulf and adjacent seas there are some noteworthy remarks to be made on the as yet incomplete qualitative and quantitative study of the East Beach Lagoon plankton.



Composition of the average catch of zooplankton per month at the three collecting stations in the East Beach Lagoon (numbers are per cubic meter of water filtered).

Details on field collecting methods and station layout can be found in the report on the Ore Dike Project. Samples taken from the three stations, one at the head end, one at mid-length, and one just inside the mouth of the lagoon, have been examined about twice weekly beginning with the end of October 1957, by means of standard analytical methods. The species content resembles that of semi-enclosed brackish waters in Louisiana, Mississippi and Northwest Florida, three brackish-water copepods predominating and about 10 others typical of open coastal waters appearing on occasion at the mouth end of the lagoon. Barnacle nauplii have been the most numerous organisms in the collection thus far.

With regard to the quantity of organisms the resemblance to other Northern Gulf areas is considerably lessened. The displacement volume of the catch has remained much below 1 cc per cubic meter of water filtered throughout the period of the survey. Numerically the various organisms are also deficient when compared to other areas. For example, in the fall and winter period, estuarine waters in Marco, Alligator Harbor, and Panama City, Florida support on the average more than 10,000 copepods per M^3 , whereas in the same period the East Beach Lagoon at Galveston has averaged about 1,000 copepods per M^3 . Either the lagoon is not as suitable an environment for species that spend their entire life cycle freely suspended in water (holoplankton), or the collecting methods do not sample the population in a uniform manner. This question is of more than routine interest. If the environment is marginal for holoplankton animals the reduced competition for food and possibly other factors (oxygen, space, etc.) may be a partial explanation of why semi-enclosed brackish shallows along the Texas coast are such successful nursery grounds for penaeid shrimp despite the absence of bountiful land drainage such as in the Mississippi River system. Comparing the lagoon with open coastal water just off the beach reveals that the latter is much richer in number and variety of holoplanktonic species and that they are typically much larger in size.

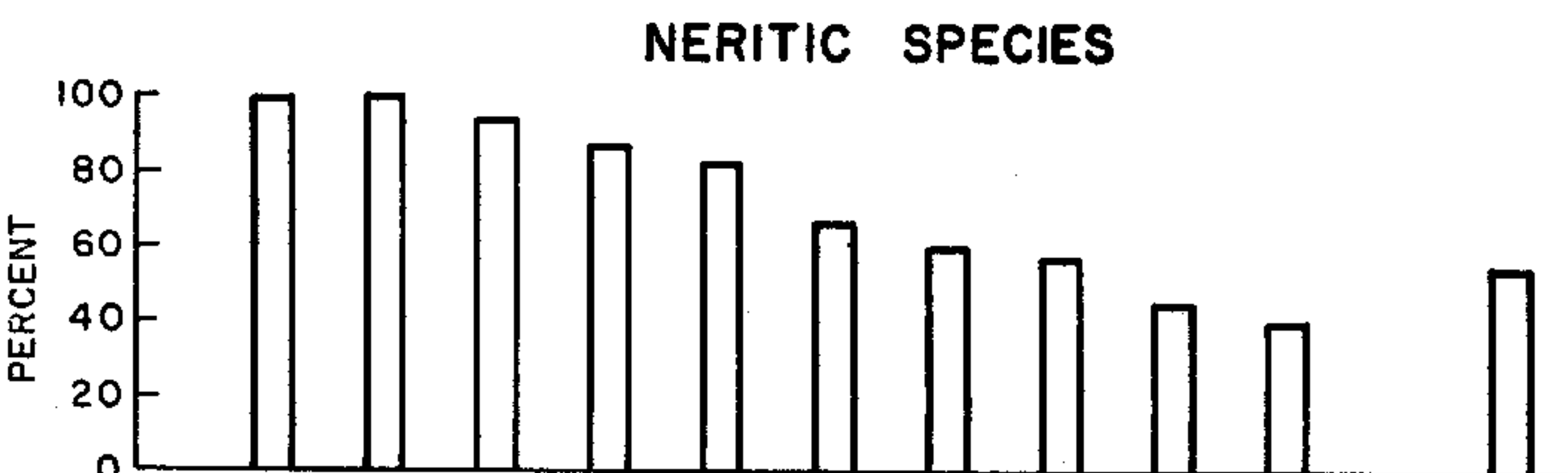
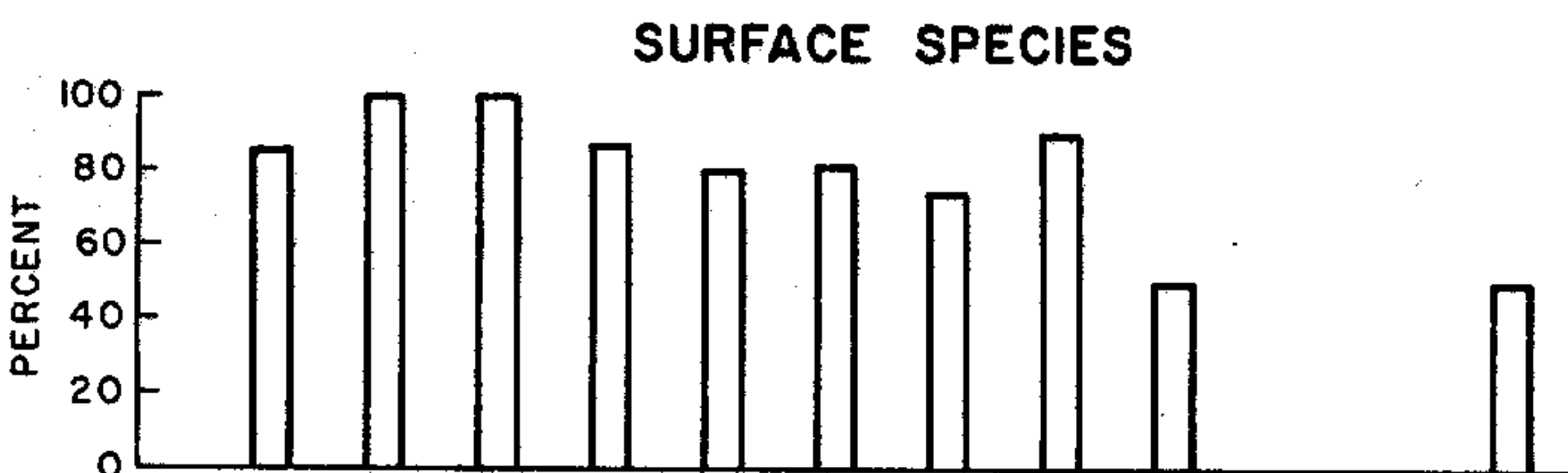
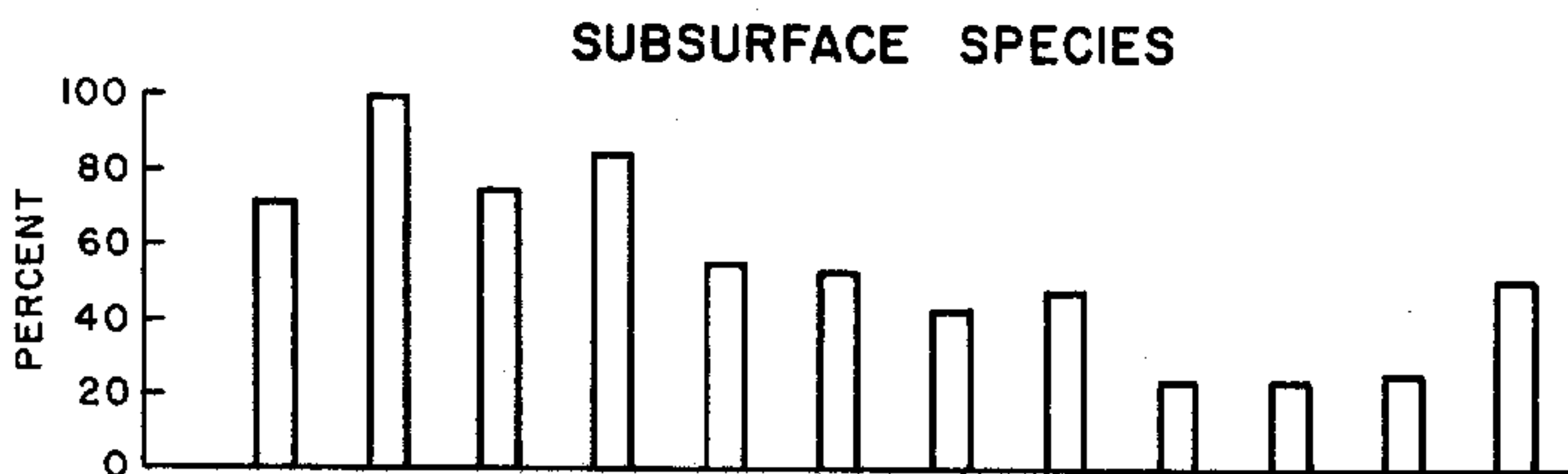
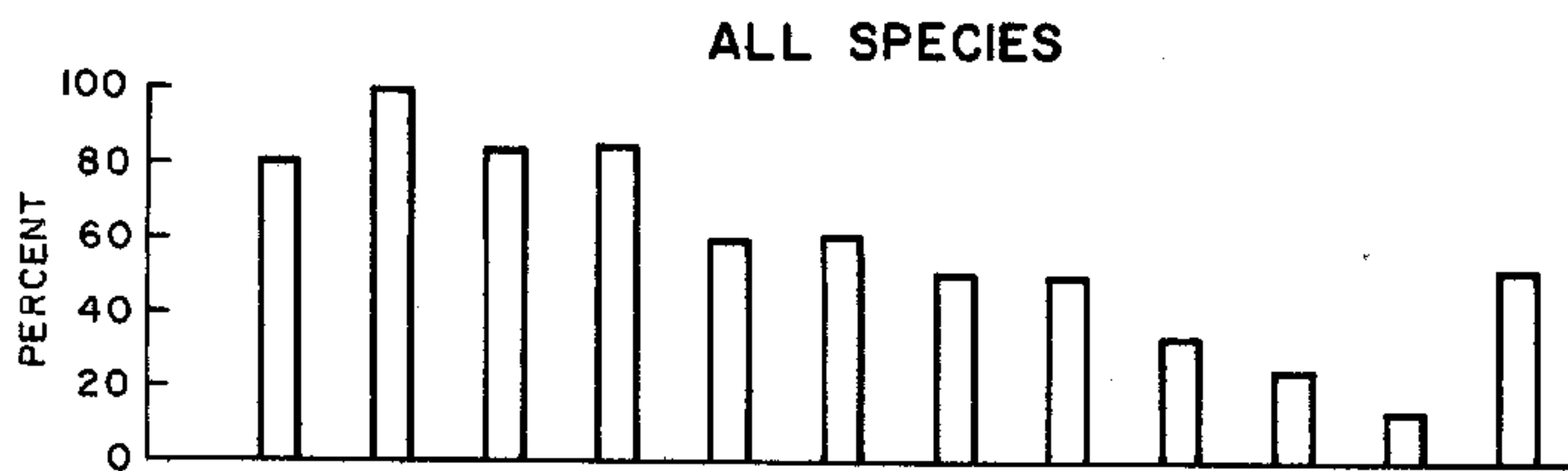
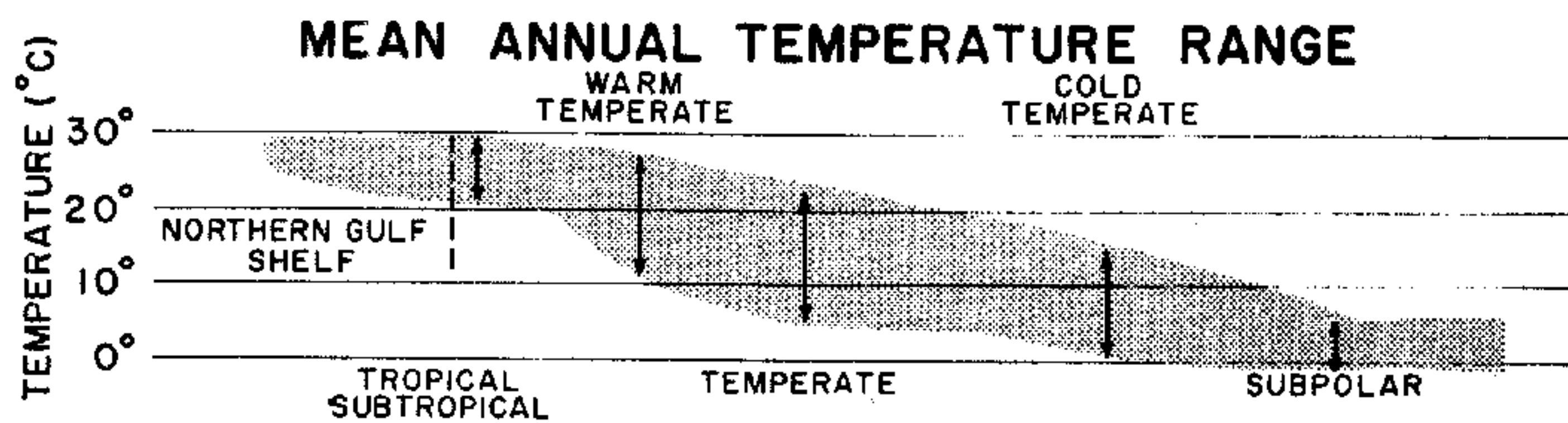
The monthly mean number of organisms taken at the three stations are shown on the accompanying graph. Fluctuations in barnacle nauplii can be seen to govern the magnitude of the standing crop at any given time. The greater number of nauplii at the month end of the lagoon apparently results from the fact that the sea wall and concrete embankments provide the only extensive surface areas suitable for adult barnacle development. The copepod population and the remainder of the catch, mostly crustacean, molluscan, and annelid larvae, show only modest variation from month to month and between stations 1 and 2. The greater abundance of copepods at station 3 is noteworthy. At this station the frequent appearance of open water copepods is unmistakable evidence of plankton recruitment from outside waters. One copepod of numerical importance in the lagoon

and in open coastal waters, *Acartia tonsa*, has two very distinctive morphological forms which differ in total length and, for want of a better word, extent of robustness. What evidence is available shows that larger, more highly developed individuals have matured in open coastal water whereas the stunted individuals matured in semi-enclosed embayments under marginal conditions. These observations suggest that in protected shallows and marshes along the coast good shrimp production and poor plankton production are directly related phenomena.

The combined warning trend in northern latitudes and the unsettled hydrographic relationship of the Gulf of Mexico to the major oceanic currents of the western North Atlantic, circumstances which have so profound an effect on Atlantic fisheries, make pertinent the question of whether plankton animals may be used with profit in better understanding these phenomena. Using the free-swimming copepods a comparison has been made between the Gulf's fauna and that of its neighboring seas, the western North Atlantic and the Caribbean Sea. In doing this it is assumed that the 135 species of calanoid copepods known from the upper 200 meters of the Gulf are biogeographically representative of the remaining pelagic fauna. Supplemental to this is an evaluation of the relationship between the Gulf and the world's oceans based upon the number of copepods in common between the former and various geographical portions of the latter. Species lists representing the Caribbean Sea to the Arctic Strait and geographical ranges of the Gulf species have been compiled from the literature and from unpublished data. In considering the western North Atlantic, Bermuda and Iceland were set as the eastern boundaries and Davis Strait and Florida Strait, the north and south limits, respectively. The species were categorized as to the most obvious of environmental types (oceanic or neritic; subsurface or surface). Such a scheme cannot avoid being arbitrary at this stage. It is, however, consistent with the known facts and permits greater accuracy in defining the quality of the exchange between adjacent hydrographic systems.

Starting first with the distribution in the western North Atlantic the ratio of species occurring at a locality that are also found in the Gulf to the total fauna of the locality was estimated as a percentage and is shown on the accompanying histogram. It should be borne in mind when considering the figure that faunistic changes in the pelagic environment are usually characterized by differences in species and in rarer instances, genera, in contrast to the much stronger taxonomic differences (genera and families) between different terrestrial fauna.

Examination of the faunal relationships shows that the number of Gulf species in common with the western North Atlantic suffers progressive reduction proceeding northward. With regard to all environmental



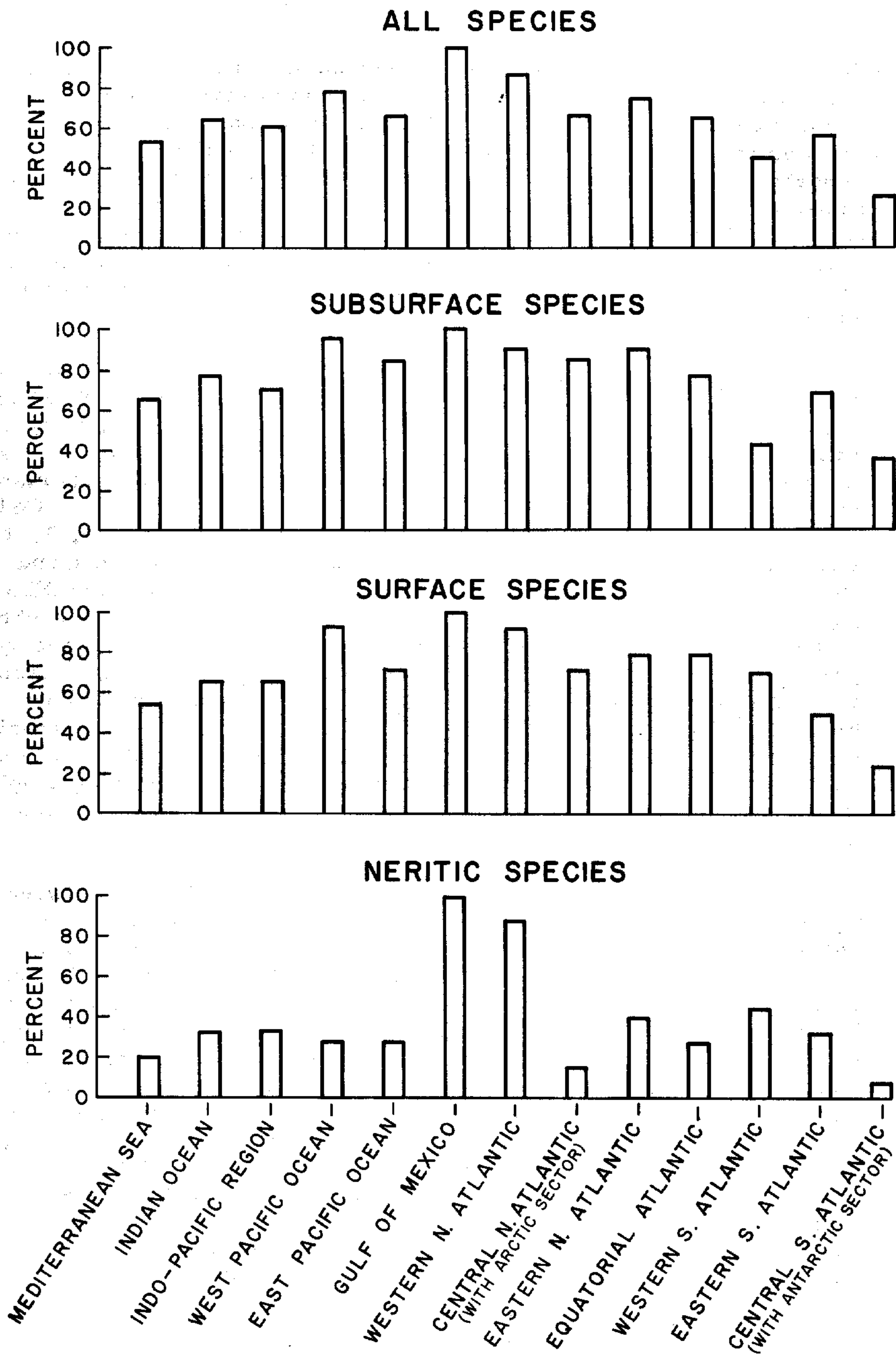
CARIBBEAN SEA
GULF OF MEXICO
FLORIDA STRAITS
CAPE HATTERAS
BERMUDA
CHESAPEAKE BAY
CAPE COD
GULF OF MAINE
GULF OF ST. LAWRENCE
GREENLAND-ICELAND
LABRADOR SEA-HUDSON BAY
WESTERN NORTH ATLANTIC COMBINED

Ratio (%) of calanoid copepods (in common with the Gulf of Mexico) to the fauna known for various portions of the Caribbean Sea-western North Atlantic system and mean annual temperature range of surface waters over this range.

types of copepods decrease in the number of Gulf species and corresponding increase in the number of non-Gulf species in varying combinations are most conspicuous between contiguous areas, Cape Hatteras-Bermuda, Cape Hatteras-Chesapeake Bay and Gulf of Maine-Gulf of St. Lawrence to the north. This seems to be most uniformly expressed in the subsurface species group. The surface species group is fairly uniform up to the Gulf of Maine-Gulf of St. Lawrence division where a decisive change is seen. The neritic species show a more gradual change between contiguous areas although the most pronounced breaks coincide with the general pattern observed in the oceanic species.

The three faunistic regions, 1. Caribbean Sea-Cape Hatteras, 2. Cape Hatteras -Gulf of Maine and 3. Gulf of St. Lawrence-Davis Strait, suggested by this preliminary analysis, clearly outline the major hydrographic features that govern conditions off eastern North America; namely 1. the Florida Current between Florida and Cape Hatteras (Carolinian province), 2. Middle-Atlantic region from Cape Hatteras to Cape Cod (Virginian province), and north of the transitional Gulf of Maine, 3. the Labrador Current in the region between the Gulf of St. Lawrence and Davis Strait. That the current systems do not in themselves constitute physical barriers is amply shown in the continuity of so large a number of species over the entire range in question. Closely related to the hydrographic changes, however, are differences in temperature more meaningfully considered in terms of the mean annual range. Conspicuous breaks in the faunal composition, then coincide with shifts in the yearly temperature range which in turn correspond to the temperature ranges widely recognized as exemplifying those inhabited by tropical-subtropical, warm-temperate, temperate, cold-temperate (boreal) and subarctic-arctic communities.

Another method of analysis whereby the relationship between contiguous regions were compared irrespective of any single fauna (the so-called coefficient of community index plotted in a trellis diagram) as well as disregarding environmental types of the various species has been tested. It clearly shows that in the north-south gradient differences between any contiguous pair of localities are of the same order of magnitude. This can be considered as resulting from the looseness of barriers in the pelagic environment permitting occurrence of extensive mixing of faunas. It points out that limitations to this preliminary account include 1. ignoring seasonal data on the distribution of species, 2. differences in the sensitivity to change between species, 3. the uncritical use of all available records disregarding whether the records represented expatriated species or species taken within their normal range. It is worthy of mention that several species can be singled out as apparently characterizing each faunal region. Also with the use of



Percentage of calanoid copepods in the Gulf of Mexico that occur in other portions of the world.

the faunal lists an opportunity is available to estimate thermal sensitivity of many of the species from the regions under consideration. Although the details are better reserved for a forthcoming technical report, those species appearing to react to a narrow range of ecological conditions could become useful hydrographic and ecological tools serving to indicate distinctive types of conditions.

A rough estimate has been made of the faunal relationship between the Gulf of Mexico and the world's oceans based on the percentage of calanoid copepods that the former and various geographical entities of the latter hold in common. Limitations in our knowledge of these organisms and variation in the completeness of faunal lists representing various parts of the world make this account provisional. However, it is a necessary beginning in the development of a modern interpretation of plankton animal biogeography and evolution. Comparisons include the total fauna as well as separate consideration for the more basic environmental types of species. As expected the percentage of species in common between the Gulf and other regions appears to vary directly with geographical distance. Similarity is highest in the subsurface group, very much the least in the neritic group. The close relationship shown between the Gulf and the western North Atlantic is expected but the strong affinities for the western Pacific Ocean is surprising. This is especially true since the intervening regions either to the east or the west show the same percent of faunal similarity and they have from 10-20 percent fewer species in common with the Gulf than has the west Pacific fauna. In the light of recent inferences made in the literature questioning the existence of truly neritic free-swimming copepods it should be mentioned in passing that from this analysis neritic copepods are very much a reality and must be taken into account in studies of plankton communities.

As a service to other institutions and in exchange for their aid, reference collections of identified copepods from the Gulf of Mexico have been deposited with Dr. Gordon Gunter, Gulf Coast Research Laboratory, Ocean Springs, Mississippi, Dr. Robert Hutton, Florida State Board of Conservation, St. Petersburg, Florida, Dr. Jose A. Suarez, University of Villanueva, Havana, Cuba, and Dr. K. K. Tiwari, Zoological Survey of India, Calcutta, India.

NUTRITION OF RED TIDE ORGANISMS

W. B. Wilson and Sammy Ray, Project Leaders

At the beginning of the current year, we were able to grow bacterized cultures of Gymnodinium breve in an artificial sea water medium, but this medium would not support bacteria-free cultures. Since the dominant bacterial colony type in the bacterized cultures was a producer of vitamin B₁₂ active substances, we tested extracts of materials which contain a variety of vitamin B₁₂ analogues. Extracts of soils, compost, and bovine feces were tested. In addition, we tested extracts of river water, bacterized cultures of G. breve, aged sea water of a G. breve bloom, and vitamin B₁₂ analogues. All of the above substances except the vitamin B₁₂ analogues promoted growth of bacteria-free G. breve cultures if added to the artificial sea water medium. Additional tests were conducted to determine the active substance(s) common to these materials, however, these tests were discontinued after the use of a mixture of trace elements proved successful.

The results of tests of nine organic complexes such as tryptone, peptone, trypticase, and yeast extract were similar to the results of tests of extracts for possible vitamin B₁₂ analogues. In an attempt to determine the growth promoting substance(s) of these complexes, we tested 16 amino acids, singly and in groups. The amino acids did not improve growth in the medium and cysteine rendered the medium toxic. The tests were discontinued also after the use of a mixture of trace elements proved successful.

Tests of various combinations of trace elements, resulted in an artificial sea water medium which supports good growth of bacteria-free G. breve cultures. The cultures have been subcultured several times without diminution in growth. The mixture of trace elements was developed from tests which have extended over the past two years. The results of these tests emphasize the possible importance of trace elements and substances which affect the balance of these elements in the development of natural blooms of G. breve.

After we developed the medium shown, tests of the need by G. breve for the various components of the medium were initiated. These tests are still in progress as only preliminary tests of some of the components have been completed to date. The following is a summary of the results of the completed preliminary tests.

Components	Conc./L of Distilled Water
Sodium chloride	30.0 grams
Potassium chloride	0.6 gram
Magnesium chloride	4.5 grams
Magnesium sulfate	6.0 grams
Calcium chloride	0.7 gram
Potassium phosphate	1.0 milligram
Potassium nitrate	1.0 milligram
Vitamin B ₁₂	1.0 microgram
Biotin	0.5 microgram
Thiamine	10.0 milligrams
Metals Mixture U II *	10.0 milliliters
Tris(hydroxymethyl)aminomethane	0.4 gram
EDTA (disodium salt)	10.0 milligrams
Potassium nitrite	1.0 milligram

*METALS MIXTURE U II

Components	Milligrams /100 milliliter
EDTA (disodium salt)	15.0
Iron (chloride)	0.1
Copper (cupric chloride)	0.1
Manganese (manganous chloride)	1.0
Zinc (chloride)	0.5
Nickel (chloride)	0.1
Aluminum (chloride)	0.5
Cobalt (chloride)	0.5
Rubidium (chloride)	1.0
Barium (chloride)	0.1
Selenium (selenic acid)	0.5
Vanadium (ammonium vanadate)	0.1
Titanium (oxide)	0.5
Zirconium (chloride)	0.5
Chromium (potassium dichromate)	0.5
Strontium (chloride)	0.5
Molybdenum (sodium molybdate)	0.5
Boron (boric acid)	0.5
Cesium (chloride)	0.5
Cerium (cerium ammonium nitrate)	0.1
Cadmium (chloride)	0.1
Tin (chloride)	0.1
Ruthenium (chloride)	0.1
Rhodium (chloride)	0.1

An artificial sea water medium for the growth of
bacteria-free culture of Gymnodinium Breve.

An artificial seawater medium for the growth of bacteria-free
cultures of Gymnodinium breve.

Vitamins

Vitamin B₁₂ and its analogues was not required for growth of bacteria-free G. breve in this medium. Vitamin B₁₂ may be a contaminant in some of the chemicals used, if so, there is sufficient present because additions of various amounts of vitamin B₁₂ did not increase growth significantly.

Thiamine and biotin are necessary for growth of G. breve in this medium. If these vitamins are employed separately, biotin does not promote growth and thiamine effects only a limited increase of organisms. However, if both are added to the medium, good growth results are obtained. The growth response to thiamine and biotin has not been proportional to the amount of these substances added. This response indicates that thiamine and biotin may condition the medium and not function as vitamins as suggested by Droop. Of course, other presumptions are just as probable, but each is based partly or wholly on speculation until additional work is completed.

Inorganic Nutrients

G. breve requires phosphorus for growth in this artificial medium, but the absolute amount needed for growth has not been determined because the major salts contain phosphorus as an impurity. Cultures grew well without adding phosphorus until we recrystallized the sodium chloride to increase its purity. Increasing the phosphorus additions above the minimum amount necessary for growth does not increase the number of organisms. The addition of 0.1 microgram atom of phosphate phosphorus per liter (0.1 μg at P/L) is sufficient to support good growth. Chemical analyses of the medium for total phosphorus with this amount added indicate that it contains between 0.1 and 1.0 μg at P/L. Therefore, the amount of phosphorus required for maximum growth in this medium is apparently about 1.0 μg at P/L or less. The development of a medium which will sustain a higher number of organisms and the use of higher purity chemicals may afford a better understanding of the role of phosphorus in G. breve blooms.

On the basis of tests conducted to date, G. breve does not require an inorganic source of nitrogen (nitrate, nitrite, or ammonia) for growth in this medium. In these tests, cultures have grown well following four serial subcultures without additions of inorganic nitrogen. These results indicate that either G. breve utilizes organically the nitrogen of the thiamine, tris hydroxymethylaminomethane (TRIS buffer) or EDTA; or that there is sufficient inorganic nitrogen present as a contaminant in the major salts of the medium; or that G. breve can utilize

atmospheric nitrogen. Rice stated during the Red Tide Symposium that he had found that EDTA is metabolizable. The growth response to thiamine could be the fulfillment of a need for an organic nitrogen source and some of our experiments indicate that tris buffer may have other functions besides buffering pH.

Preliminary experiments were conducted to determine the growth response of G. breve to the individual elements of Metal Mix VII. To date, the results are inconclusive, but copper, titanium, zirconium and zinc appear to be beneficial.

Organic substances

Various carbohydrates, amino acids and organic complexes were tested to determine if they would improve growth. Although G. breve grew well in media containing many of these substances, the growth was no better than it was without them.

In spite of our success in developing an artificial medium which supports bacteria-free cultures of G. breve, we are not obtaining standard survival and growth in replicate cultures. Therefore, replicates must be used in each experiment. Even so, some results are of doubtful value. Because of this unreliability, studies to improve and standardize the technique for culturing G. breve have been continued. The suitability of a commercial detergent known as 7X (Linbro Corporation, New Haven, Conn.) which is recommended for use in tissue culture was tested. The growth of G. breve in four different culture media in 7X-cleaned culture vessels and in regularly-cleaned vessels was compared. The regular cleaning procedure consists of washing in "Alconox" and rinsing in hot, 7 per cent nitric acid. The clean glassware is pretreated by filling the culture vessels with distilled water or distilled water containing 0.03 per cent ethylene-diamine tetraacetic acid, disodium salt ($\text{EDTA} \cdot \text{Na}_2$) and then autoclaving the filled vessels at 15 pounds pressure for 15 minutes. After autoclaving, the pretreatment fluid is discarded and the medium is placed in the tubes without further treatment.

G. breve survived poorly in non-pretreated, 7X cleaned vessels--70 per cent (55 of 80) cultures failed after two weeks' incubation whereas only 17.5 per cent (14 of 80) of the cultures died out in non-pretreated, regularly-cleaned vessels. On the other hand, the survival of G. breve in pretreated culture vessels was comparable--5 per cent (4 of 80) of the 7X-cleaned vessels failed and 2.5 per cent (2 of 80) failed in the regularly-cleaned vessels. Additional tests indicate equally that 7X-cleaned, pretreated vessels are as successful as regularly-cleaned, pretreated vessels. The 7X cleaning solution will be used routinely for cleaning glass culture vessels.

COMPOUNDS TOXIC TO RED TIDE ORGANISMS

W. B. Wilson, Project Coordinator

Studies to determine the effects of various factors on copper toxicity to G. breve were continued during the past year. The effects of soil extracts, chelating agents, various exposure times to toxic concentrations of copper, and the amount of time various concentrations of copper will remain toxic in sea water were tested. In addition, tests were made on the toxicity of flourescine dye and the use of dialyzing membranes to test the effectiveness of copper in field experiments of control methods.

Soil extract in concentrations as low as 10 milliliters per liter of medium reduced the toxicity of copper. Approximately twice as much copper was required to cytolyse all the cells of a culture within 24 hours if this amount of soil extract was added. The same concentrations of Florida peat-soil extract and Caloosahatchee River water reduced the toxicity of copper, but to a slightly lesser degree.

Metal chelating agents such as EDTA and citric acid are known to reduce metal toxicity to aquatic organisms. We made tests to determine the minimum amounts of the substances that would be required to alter the toxicity of copper. The results of these tests show that as little as 0.05 milligrams of EDTA·Na per liter will reduce copper toxicity by as much as 50 per cent or more. Citric acid reduced copper toxicity in similar tests, but much more citric acid is necessary and the reduction is less.

In an effort to understand the effectiveness of copper as an algaecide in sea water with changing conditions that occur in the field, two series of tests were conducted. The first series was to determine whether or not organisms which were subjected to toxic concentrations of copper would survive if the concentration was reduced by dilution before the organisms cytolyse. The second series was to determine whether or not sea water containing toxic concentrations of copper remained toxic and if so, for how long.

The results of the first series of tests indicate that if G. breve survives until the copper concentration is reduced to a non-toxic level by dilution, they will survive for an indefinite period which exceeds 96 hours. A few organisms which remained intact in high concentrations of copper until dilution, cytolyse later, but these may have been lifeless. If these results are indicative of what will happen

in the treatment of natural blooms and such is probably the case, more copper will be required in areas of greater mixing and at times of greater mixing than is otherwise necessary to control these blooms.

The results of the second series of experiments indicate that the toxicity of copper decreased steadily from the time the copper is added to sea water until, at least, 48 hours afterward. The results of these tests indicate that four to five times more copper sulfate would be required if it does not come in contact with the organisms until 48 hours after the algaecide is placed in sea water (0.04 to 0.05 ppm compared to 0.2 ppm). If this is the case, a mass of sea water treated with copper sulfate will remain toxic for a short and limited period depending to some degree on the rate of application.

Tests on the toxicity of flourescine dye to G. breve indicated that it is not toxic in concentrations of 100 parts per million. These results indicate that flourescine dye can be used as a marker in field experiments without altering the results.

During the past year, we attempted to establish methods of testing copper toxicity in the field by using laboratory cultures enclosed in dialyzing membranes and placed at specific field stations. We have not tested this method in the field to date as more refinement is needed. Our early tests in the laboratory were unsuccessful because the membranes we employed contained toxic substances. These toxic substances were reduced by filling the membranes with distilled water and autoclaving them immersed in water. Subsequent tests show that the copper passed through the membrane and killed the organisms, but more time was required than for those on the outside. A more permeable membrane may alleviate this condition somewhat, but more testing is needed to determine if this method is usable.

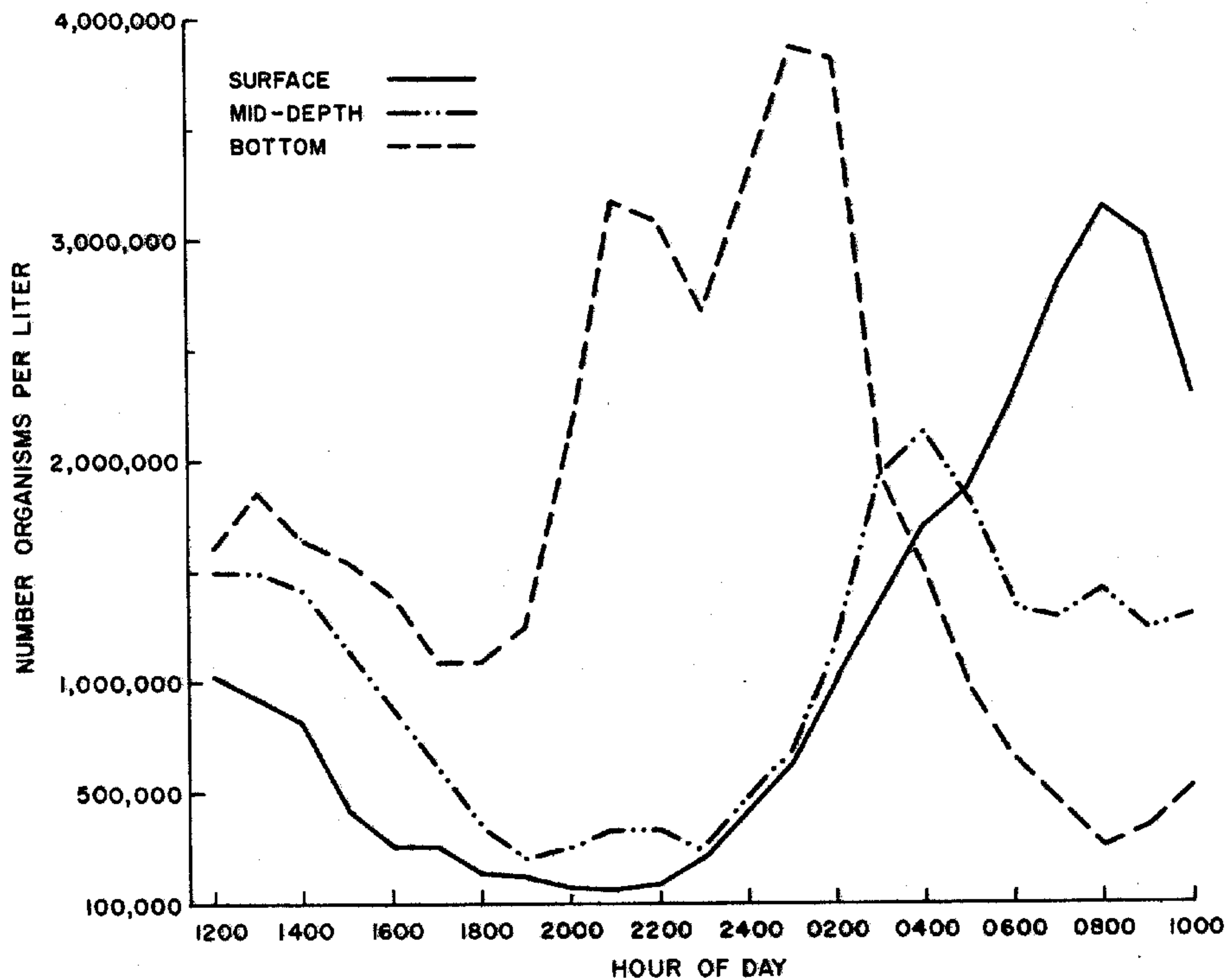
OCCURRENCE OF RED TIDE ORGANISMS

John H. Finucane, Project Leader

The red tide organism, Gymnodinium breve, is present throughout the entire year off the Gulf Coast of Florida from Tarpon Springs southward, and there is good evidence that the organism is native to the entire Gulf of Mexico. Concentrations of the organism in fish-killing density have, so far, been found mainly in the neritic areas adjacent to major river drainages. The slope of the West Coast Florida continental shelf is very gentle, so that a depth of 60 feet lies 25-45 miles offshore; consequently, the neritic zone is broad.

During periods of relatively light concentrations, tremendously dense sampling is necessary to validate the presence of the organism. For example, on two different occasions, samples taken every hour in the same water mass for periods of twenty-four hours showed the organism present in but 3 out of 50 samples in one instance, and in but 2 out of 48 in the other. Even during periods of heavy concentrations associated with fish kills, when samples were in an area of dying fishes, the counts varied from 0 to over 1 million per quart, further indicating the extreme patchiness of the organism's distribution in sea water.

On the present basis of limited evidence - two series of samples made hourly for periods of 24 hours - the red tide organism appears to concentrate at the surface during daylight hours, and to move downward at night. We plan further study of this apparent diurnal migration. The figure portrays the results of a 24-hour study conducted on October 15-16, 1957 one mile west of St. Petersburg Beach in 20 feet of water. At that time bloom stages of red tide were present and fish kills occurred during the sampling period. Weather conditions were ideal with a clam sea and cloudless sky.



Diurnal change in depth distribution of the red tide organism.

HYDROGRAPHY RELATED TO RED TIDE

Alexander Dragovich, Project Leader

Copper concentrations of Florida West Coast waters

Copper, of various substances tested in the Galveston Laboratory of the Service, proved most toxic in low concentrations to the red tide organism, Gymnodinium breve. Laboratory experiments indicated that the concentration of copper required to kill the organism ranges between 0.79 and 1.58 microgram atoms per liter ($\mu\text{gat/L}$), and suggested that copper might be the controlling factor in the life cycle of G. breve under natural conditions. As a consequence, the natural distribution of copper in waters associated with Florida red tide was studied between February 1955 and June 1957. The study area extended from Anclote Key to Marathon Key (Florida Keys) and included also the drainage systems of the Florida West coast out to the 10-fathom line. Concern was also given to the possibility of raising the copper level in the potential red tide water to the toxic level by addition of copper sulfate or other copper-containing substances, should that prove to be practical.

The copper concentrations in 2309 samples of surface and bottom waters collected from 87 stations located throughout the study area ranged over a period of 29 months from 0.00 - 0.95 $\mu\text{gat/L}$ with an average of 0.14 $\mu\text{gat/L}$. The average surface copper value of 0.15 $\mu\text{gat/L}$ was higher than the bottom average value of 0.12 $\mu\text{gat/L}$.

Considering the fact that the rivers are constantly bringing soil particles from the drainage areas into the adjacent Gulf waters, the entire investigation area is divided into four general sub-areas as follows:

1. Rivers and Canals
2. Bays and Passes
3. Gulf waters from shore line out to the 5-mile line.
4. Gulf waters from 5-mile line offshore out to the 10-fathom line.

According to this division the monthly copper average results for surface and bottom waters make it apparent that there is no seasonal trend in copper values as observed by other investigators. The greatest average copper value for the entire period of this investigation was found in the area between the shore line and 5-mile line. Comparing

the copper results from individual stations in different geographical locations within the entire area, there was not a specific station where low or high copper values were found at all times. Instead, variations in copper values were observed throughout the area and throughout the year. Exemplifying these variations are results from August 1955, when most of the stations were sampled more than once a month and the monthly range of copper values in all the bay stations was 0.00 - 0.79 $\mu\text{g/L}$ being the same as the range found at a single station at Goodland in the Everglades.

The greatest fluctuations in copper values were observed in Gulf waters, indicating an erratic distribution of copper in the Florida red tide waters, which was further verified by concentrated studies. A patchy distribution of copper in sea water was also observed in the area 65-70 miles offshore, (at depths of 270 feet) where 23 surface and bottom results ranged from 0.01 to 0.24 $\mu\text{g/L}$.

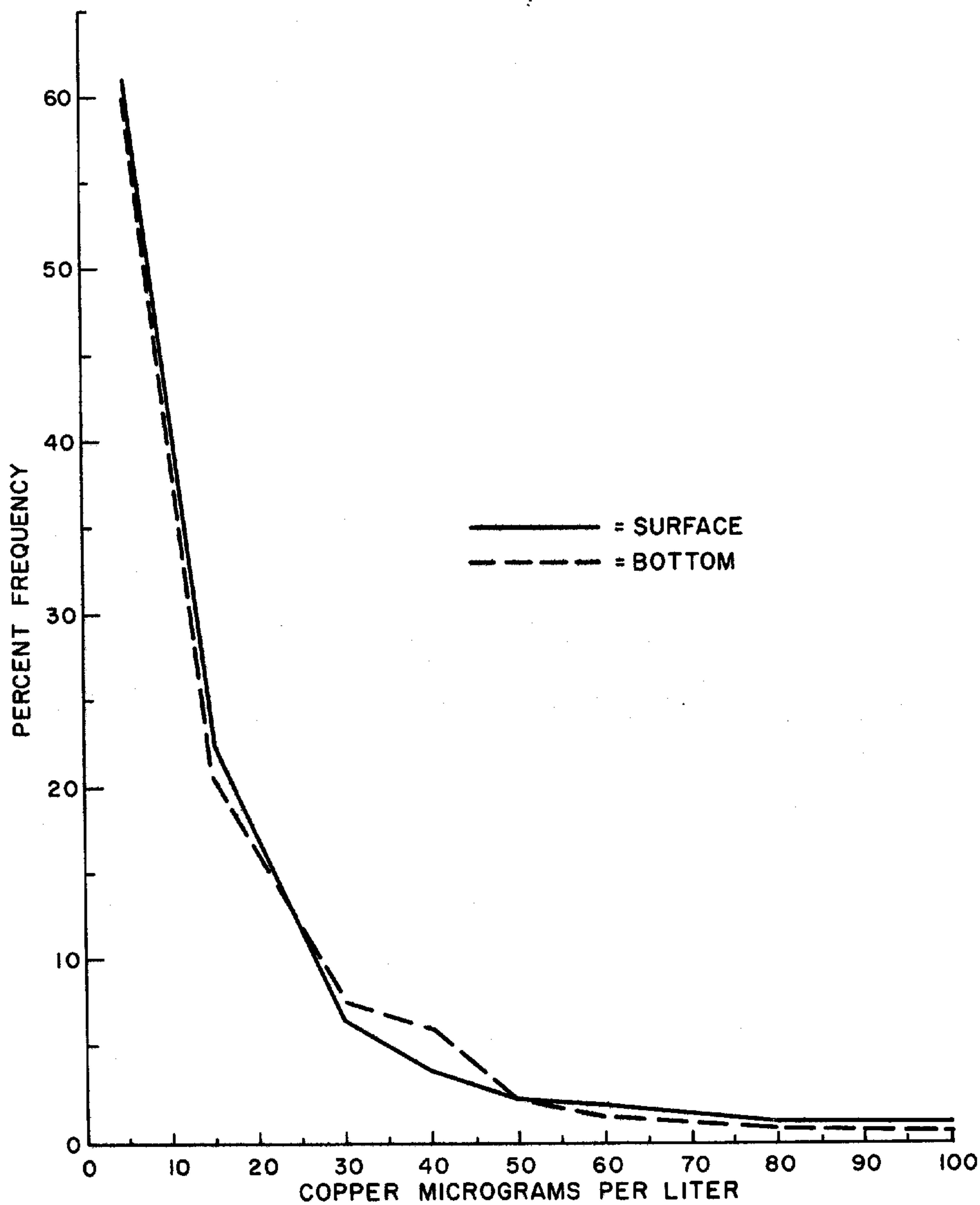
These variations in copper values indicate that copper is not homogeneously distributed in sea water. At the present state of our knowledge, these fluctuations cannot be explained satisfactorily.

Of 1884 copper samples collected in locations where the red tide organism has been found at one time or another, only in 0.7 per cent of the samples was the copper concentration within the lethal range for the organism; 99.3 per cent were harmless according to the toxicity range established by laboratory experiments.

Total and inorganic phosphorus concentration of Florida West Coast waters

Phosphorus studies were made from September 1955 to June 1957 to determine the phosphorus concentrations of Florida West Coast waters and to determine whether the outflow of rivers and canals causes any changes in the phosphorus content of local Gulf waters, as a possible factor in the development of the red tide organism.

During the period of 27 months, there were 2384 water samples collected from 87 stations scattered in an area from Clearwater to the southern tip of Florida and from Lake Okeechobee throughout the drainage systems and out to the 10 fathom line. The concentrations of total phosphorus in 1438 samples varied from 0.0 to 30.0 $\mu\text{g/L}$ and the concentrations of inorganic phosphorus in 946 samples varied from 0.0 to 28.6 $\mu\text{g/L}$.

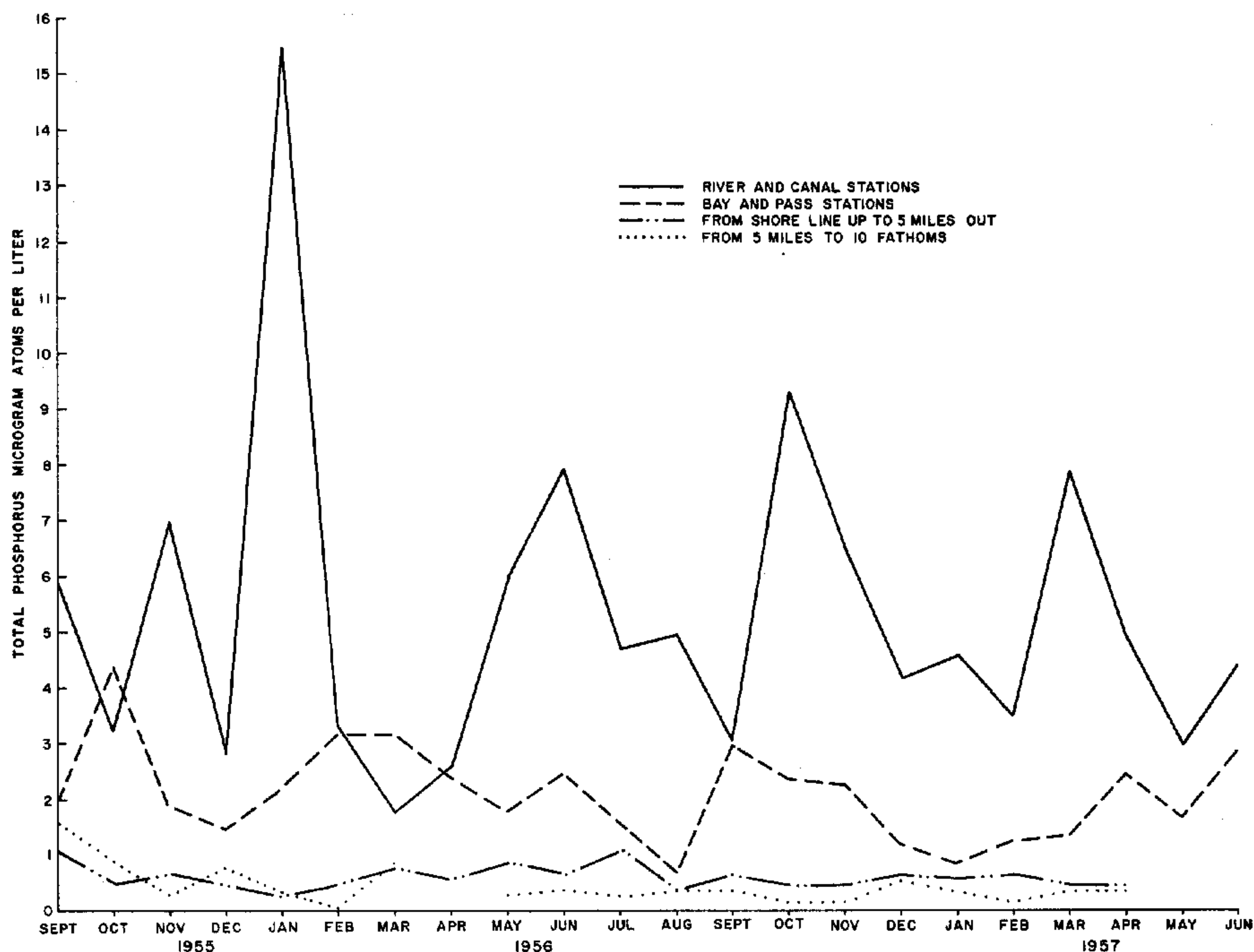


Frequency of various concentrations of copper in all samples along the west coast of Florida.

Total phosphorus concentrations generally decreased from higher values in fresher waters to low values in more saline waters. The average values of inorganic phosphorus followed the same general pattern as the total phosphorus.

The highest total and inorganic phosphorus values were found in the rivers, among which the Peace and Myakka Rivers had the highest concentrations, where values as high as 25 $\mu\text{g/L}$ were not considered unusual.

In spite of these high concentrations of total phosphorus in the Peace and Myakka Rivers, Gulf waters adjacent to Charlotte Harbor, into which these two rivers flow, were not enriched to an appreciable degree. The slight enrichment of local Gulf waters in total phosphorus was noticed at a distance 3 miles offshore in the ship channel off Boca Grande Pass where the average value of total phosphorus for the entire



Total phosphorus concentrations on the west coast of Florida.

period was 1.0 $\mu\text{g}/\text{L}$. At stations farther to the north or south of the ship channel, this slight enrichment in phosphorus was not evident, and from the 5-mile line out to the 10-fathom curve no enrichment in phosphorus was noticed.

The average value of total phosphorus in the area between the shore line and 5-mile line was 0.7 $\mu\text{g}/\text{L}$ for surface and bottom samples, and in the area between the 5-mile line and 10-fathom curve was 0.2 $\mu\text{g}/\text{L}$ for the surface and 0.4 $\mu\text{g}/\text{L}$ for the bottom.

High concentrations of total phosphorus in the rivers were caused by high inorganic phosphorus content, which exceeded the amount of organic phosphorus at all times. In the bays the inorganic phosphorus values were higher than the organic phosphorus values; and in the adjacent Gulf waters, although the fluctuations were great from one sample to another, the majority of the samples had slightly more organic than inorganic phosphorus where the data were comparable.

Nitrate-Nitrite ($\text{NO}_3\text{-NO}_2$) concentrations of various Gulf of Mexico waters

Studies were made to determine the nitrate-nitrite concentrations in the rivers, bays, and adjacent Gulf waters, and to establish possible correlations between the incidence of the red tide organism and the nitrate-nitrite concentration.

Nitrate-nitrite determinations were made of 1001 samples of surface and bottom waters collected from 87 stations in an area from Clearwater to the southern tip of Florida and from Lake Okeechobee to the 10-fathom curve between May 1956 and June 1957. The concentrations ranged from 0.0 to 38.2 $\mu\text{g}/\text{L}$.

Monthly $\text{NO}_3\text{-NO}_2$ average values according to the rivers, bays and Gulf waters demonstrate that:

- a. The highest of the $\text{NO}_3\text{-NO}_2$ concentrations are found in the rivers and the greatest addition of $\text{NO}_3\text{-NO}_2$ takes place during the late fall, winter and early spring months.
- b. September and May 1956 are the only months when the bay and Gulf $\text{NO}_3\text{-NO}_2$ averages exceeded the river values.
- c. The amount of $\text{NO}_3\text{-NO}_2$ decreases in somewhat irregular pattern from fresher to more saline waters. This decrease is not as distinctive as in the case of average total phosphorus values shown.

- d. The seasonal annual cycle is rather difficult to detect from combined data, but there is a trace of evidence for seasonal variations. The low values were observed during May and June.

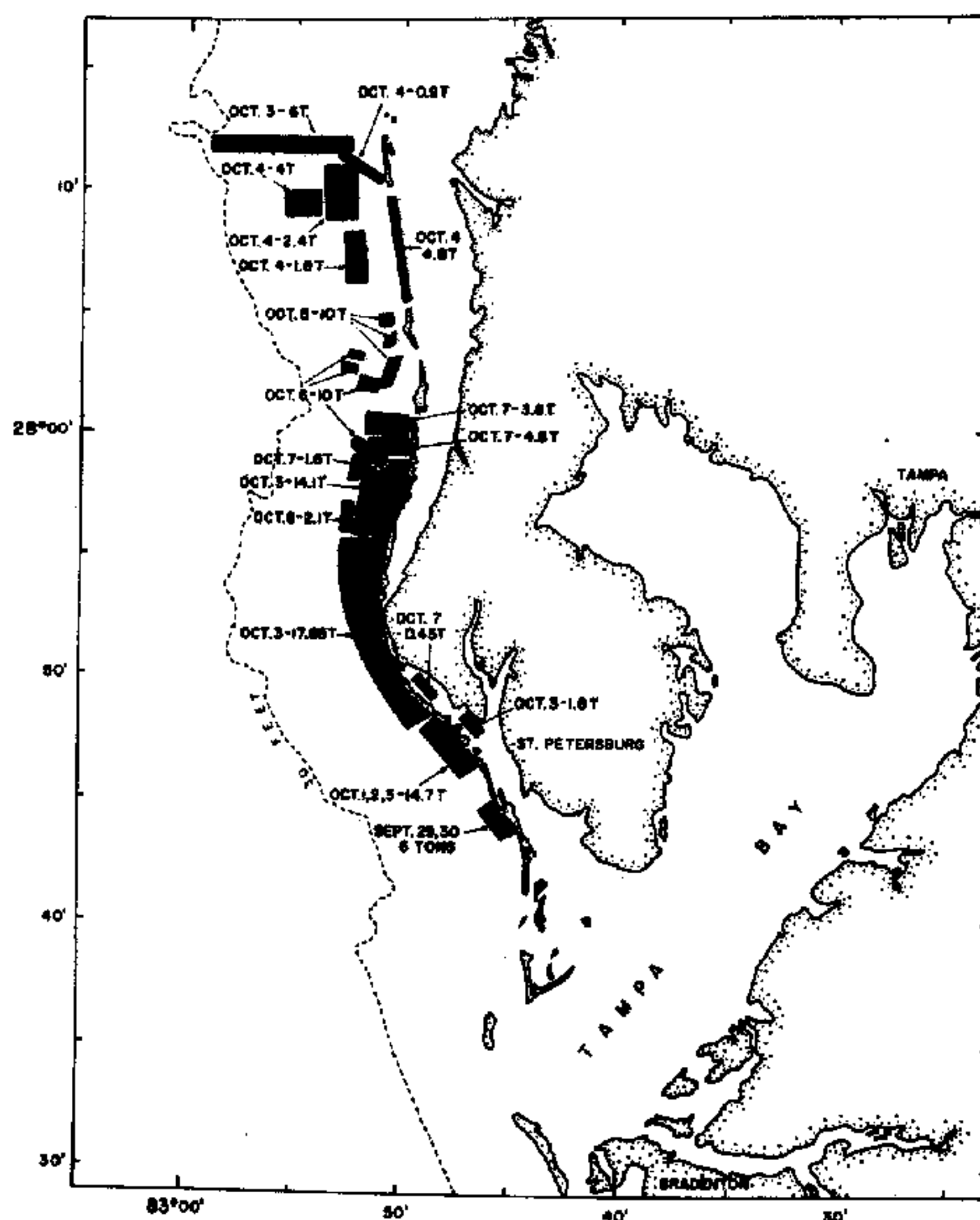
The highest average $\text{NO}_3\text{-NO}_2$ concentrations for the 14 months were observed at the stations in Myakka and Peace Rivers. The Caloosahatchee and Barron Rivers had the next highest average values.

In the offshore waters of Charlotte Harbor, Tampa Bay, Big Carlos Bay and Barron River the enrichment of Gulf waters with $\text{NO}_3\text{-NO}_2$ is evident, and generally in the rivers $\text{NO}_3\text{-NO}_2$ values were observed to be three times higher than those in the local Gulf waters and one and one-half times higher than those in the bays. In other words, there is a 3:2:1 ratio of the $\text{NO}_3\text{-NO}_2$ content of river, bay and Gulf waters. The decrease of $\text{NO}_3\text{-NO}_2$ values from the sea shore line up to the 10-fathom line was observed to be a gradual process.

LARGE-SCALE EXPERIMENTAL CONTROL OF RED TIDE

George A. Rounsefell, Director

When the red tide outbreak commenced in the autumn of 1957 it was decided to make a large-scale test to determine whether red tide could be controlled with copper sulfate. About 16 square miles stretching along 32 miles of shoreline from Anclote Key to Pass-a-grille Beach, off St. Petersburg, Florida were dusted with copper sulfate ($\text{Cu SO}_4 \cdot 5\text{H}_2\text{O}$) at about 20 pounds to the acre by crop-dusting planes.

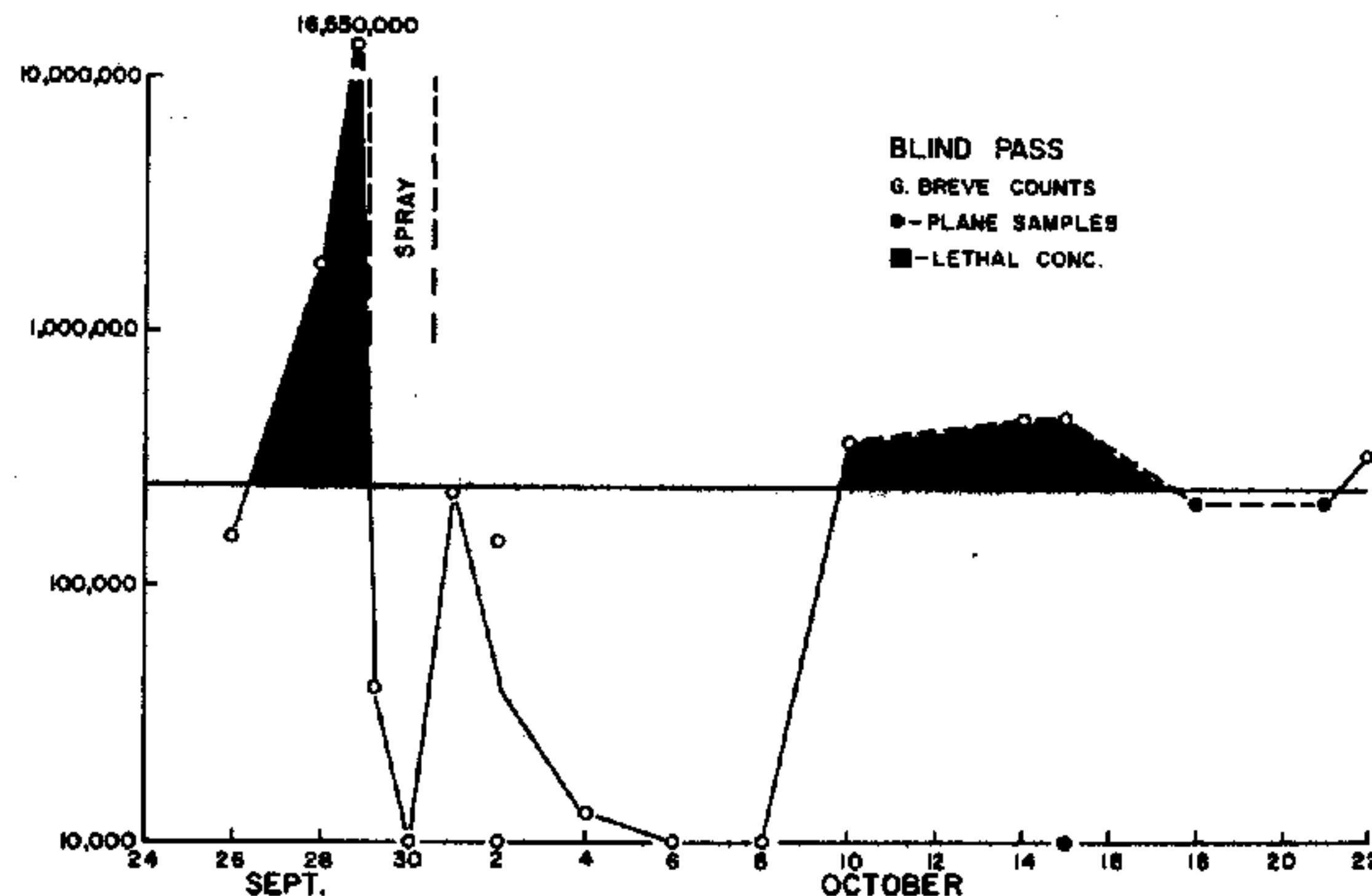


Showing the tons of copper sulfate sprayed on various areas during the experimental red tide control.

The copper quickly reduced the red tide organism, Gymnodinium breve, from several million to practically none per liter. This relieved the area of the respiratory irritation caused by the airborne toxin of G. breve.

The operation cost \$43,000 or about \$4 per acre. Although this could probably be reduced to \$3 per acre the cost for controlling a major outbreak would be prohibitive. In 2 out of 5 of the areas dusted the red tide organisms had increased to fish-killing concentrations within 10 to 14 days after dusting.

Because of the high initial cost and the temporary nature of the control we cannot recommend the use of copper sulfate except perhaps for temporary relief in local situations from the airborne toxin.



Surface water samples from off Blind Pass showing the number of red tide organisms and copper concentrations per liter. The before and after dusting points show the swift destruction of G. breve when the copper concentration rose.

COPPER ORE EXPERIMENTS FOR RED TIDE CONTROL

Kenneth T. Marvin, Project Coordinator

The red tide control program was established for the purpose of finding a practical means of discouraging the formation of the fish killing bloom that has been ravaging the west coast of Florida for many decades. A method that has been used in the past with various degrees of success on related outbreaks consisted of raising the copper concentration of an area to a high enough level to discourage the formation or continuance of a bloom.

Thus, the Japanese have been successful in dispersing fish killing blooms over very localized areas by dragging burlap sacks of copper sulphate through the affected areas. Red tide outbreaks off the west coast of Florida have been temporarily dispersed by dusting the affected area with copper sulphate from planes.

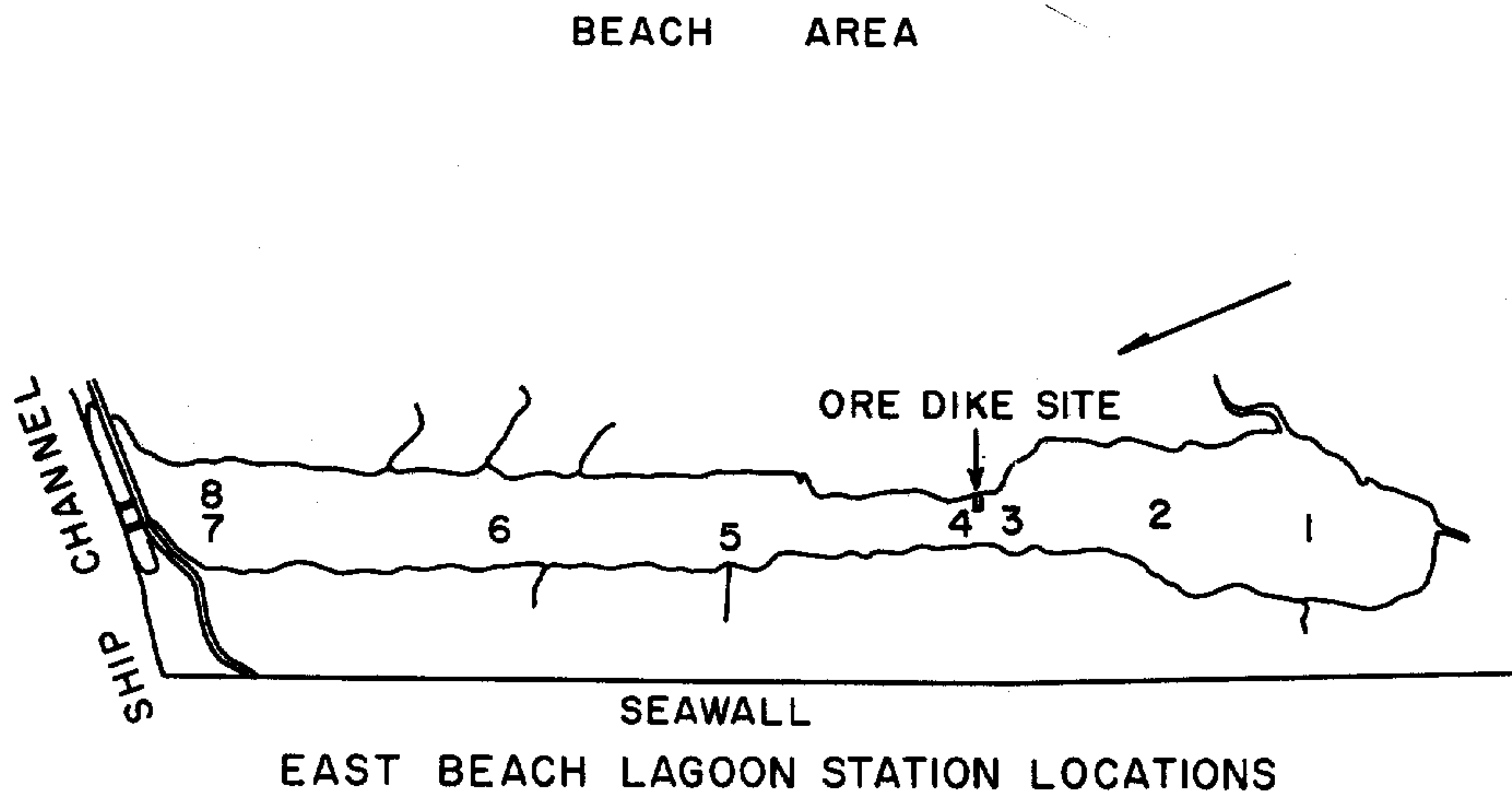
This method of controlling a bloom is probably the most effective means devised thus far. Unfortunately, its high cost makes its use economically unfeasible for long-term control. When copper sulphate is placed in sea water, it dissolves rapidly, raising the copper concentration level in the immediate area to many times that of the lethal level of the red tide organism. During this "flash action" probably all the organisms in the area are either killed, or discouraged to a point where they have no desire to bloom. In a relatively short time, however, tidal and current actions reduce the copper concentration sufficiently so that a fresh crop of organisms brought in by the tides, currents, etc., again find conditions favorable for a bloom. To be effective over long periods of time, copper sulphate must be added at relatively frequent intervals. The cost of protecting a large coastal area by this means would, naturally, be prohibitive.

To surmount this difficulty the source of copper would have to be capable of releasing a relatively small amount of copper into solution over a long period of time. With this in mind the Gulf Fishery Investigations started the ore dike program, the purpose of which was to investigate the possibility of using a copper ore as a source of copper. The particular ore under consideration is a sulphide ore containing roughly 1 per cent copper, 3.5 per cent iron and 6 per cent sulphur.

Preliminary investigations have shown that the ore in a closed system (4 pounds of ore in 120 gallons of sea water) will liberate a

maximum of about 1.5 parts per million of copper in about a day's time. This then tapers off to about 0.9 parts per million; the excess copper is presumably precipitated as the rather insoluble compound, basic copper carbonate. In this form it is possible that over a period of time it will have an adverse effect on the many forms of filter feeders, and grazers such as the shellfish, mullets, and other species that are of commercial importance.

The ore dike project will determine if the ore will adversely affect these commercially important species as well as the red tide organisms, or whether, as laboratory experiments seem to indicate, its effects will be felt by only red tide organisms. It is also possible that the results will be absolutely negative as far as any organism is concerned.



- East Beach Lagoon, Galveston Island, showing the 8 sampling stations.

The study is being conducted in a lagoon located at the eastern end of Galveston Island, about a mile long and, as can be seen, quite narrow. The narrowest point, which has been selected as the location of the dike, is about 230 feet across. Conclusions of the experiment will be based upon a comparison of the "normal" ecological conditions before and after the addition of a 60-ton copper-ore dike. A similar comparison will be made based on the normal life span of animals placed in the lagoon in live cars.

The "normal" ecological conditions will be based on chemical- and bio-assays. Chlorophyll analyses and zooplankton counts will be used as measures of productivity. The effect of the added copper on the normal *G. splendens* population which exists in the lagoon, will be indicative of its effect on the red tide organism, *G. breve*. The use of copper ore will be suggested as a means of controlling red tide outbreaks if the population of the red tide indicator organism, *G. splendens*, is reduced, provided, at the same time, there are no adverse effects on the commercially important species placed in the lagoon in live cars and also those that occur naturally in the lagoon.

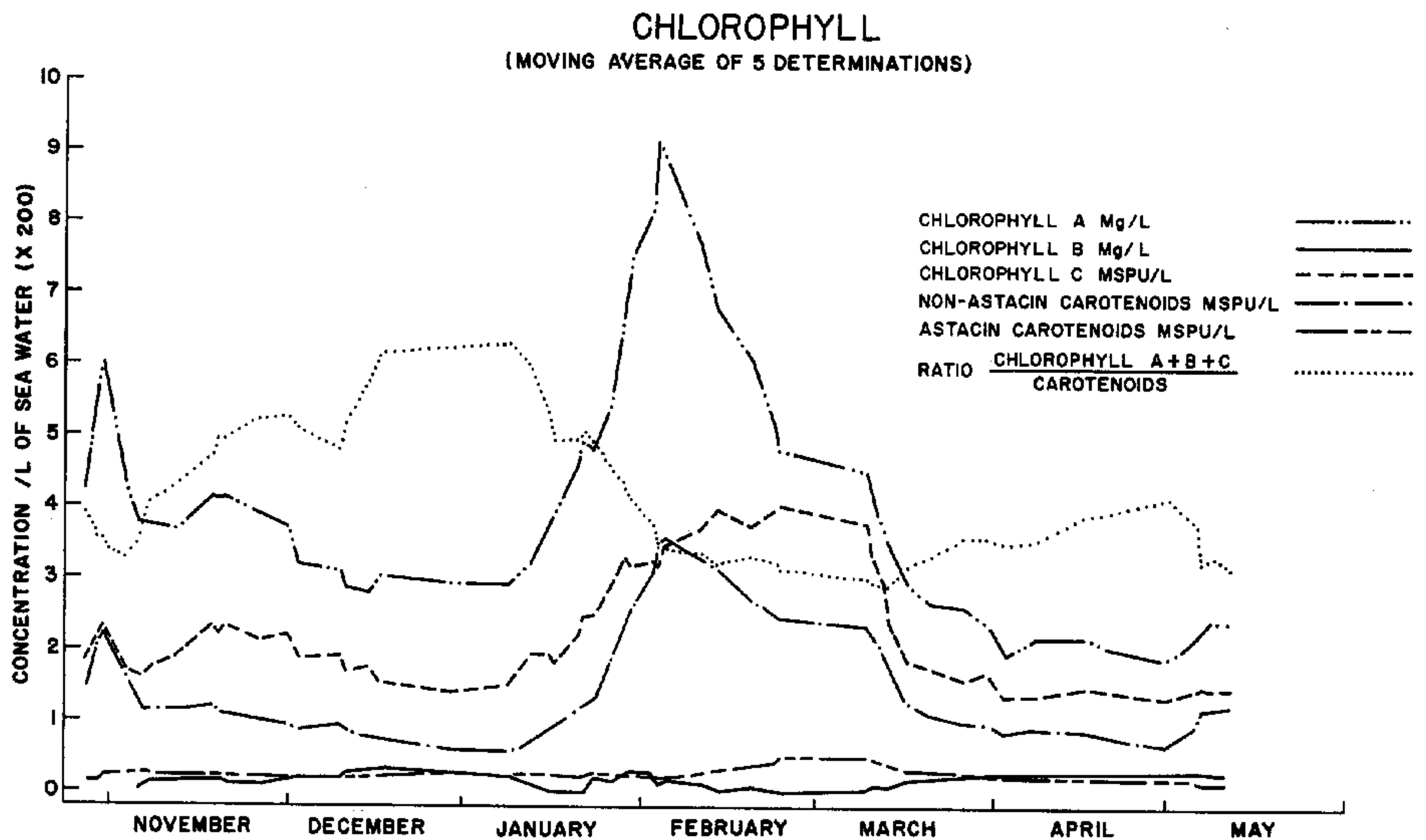
The estimate of normal ecological conditions, as was previously stated, is based on chemical and biological information. This is obtained at each of the 8 stations shown. It is also based on dip net and plankton data that has been collected during the past 5 years.

The effects of copper on the commercially important species is being investigated from three different angles. First, the effects of the basic copper carbonate on bottom feeders, etc., is being checked by exposing various species to the muddy bottom of the lagoon where it is felt that much of the basic copper carbonate will settle in spite of tidal and current actions. The normal mortality rate of these species will be determined both before and after the addition of the copper ore dike. The animals selected are shrimp, mullet, oysters, conches (*Thias*) and snails (*Littorina*). Undoubtedly there are more suitable animals, however, our selection has to be governed by the rather small selection that is available the year round. The test animals were placed on the bottom of the lagoon in 1/8-inch mesh galvanized wire live cars at each of the 8 stations. These are all checked at least three times a week. The mortality rate is based on the number of days until two-thirds of the animals are dead. When this occurs the live cars are replenished with fresh animals.

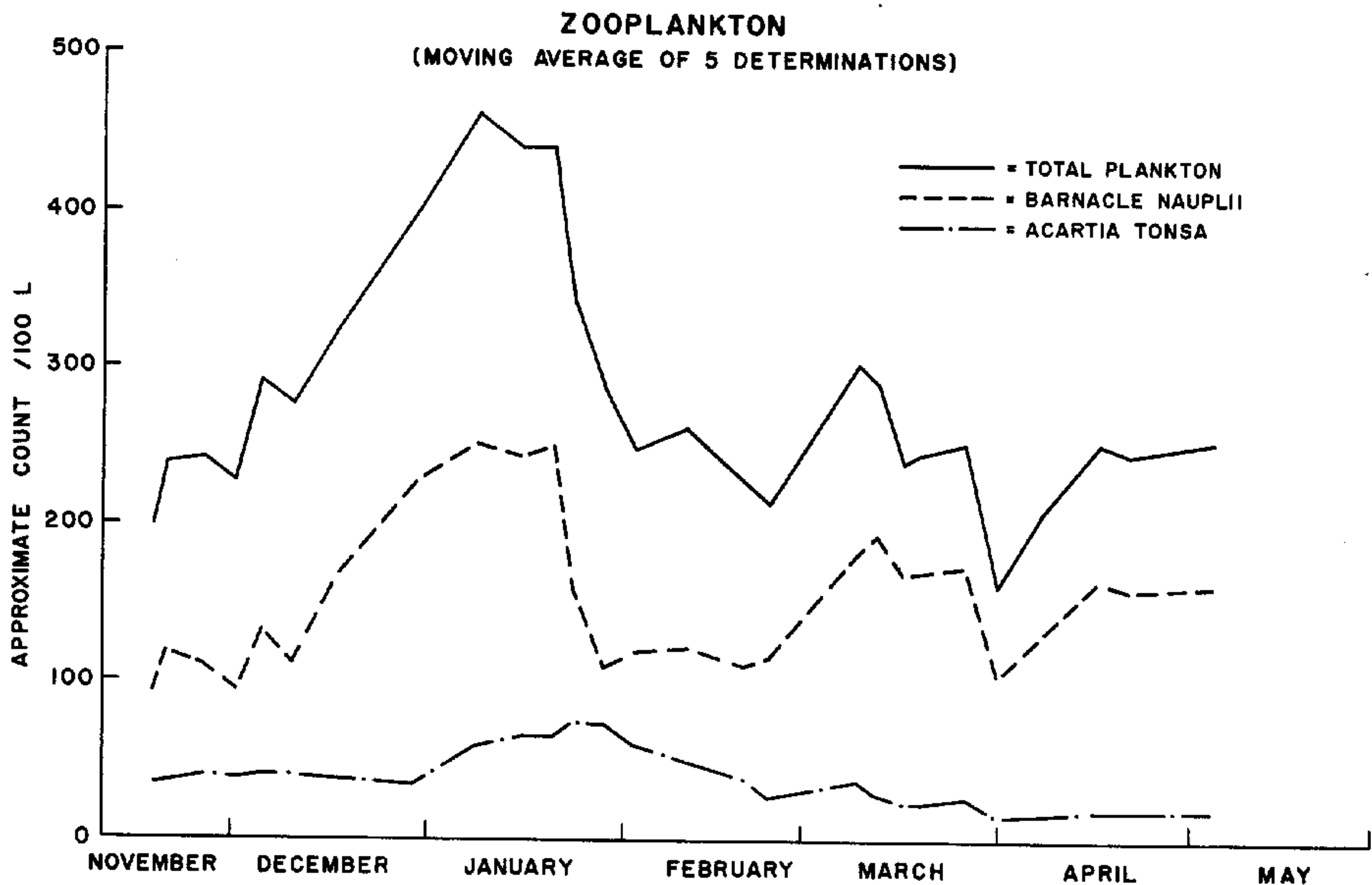
The effects of the copper in solution on the commercially important animals is being checked by subjecting the same species of test animals to the surface waters of the lagoon at each of the 8 stations.

Probably many of the live car animals die of starvation, rubbing against the screened compartment walls, etc., however, this type of variable should not affect the final results since they will be present to the same degree during both investigation periods; that is, before and after the addition of the ore dike.

A third effect the copper ore might have on the commercially important species is more or less indirect. If the copper concentration is such that the phytoplankton and zooplankton populations are



Seasonal chlorophyll concentration in East Beach Lagoon, Galveston.



Seasonal abundance of Zooplankton in East Beach Lagoon, Galveston.

either killed off or greatly reduced, the naturally existing food chain will be broken and the larger fish will seek other areas.

The indicators that are being used to measure this basic productivity, chlorophyll and zooplankton concentrations, are collected three or four times a week. During each collection trip chlorophyll samples are taken at each of the 8 stations.

A zooplankton count requires a considerable amount of time and specialized skill. Since the Gulf Fishery Investigations is limited as far as this type of help is concerned, this sample is collected at three locations instead of eight. One of these coincides with station number 1, a second is halfway between stations 3 and 4, and the third, between stations 7 and 8.

The copper liberated from the ore must be sufficient to kill or discourage the blooming of the red tide organism, G. breve. This organism does not occur in the lagoon, therefore, we are using as an indicator, its second cousin, G. splendens, which is similar to G. breve as far as the toxic effects of copper are concerned.

A count is made of this organism at each of the 8 stations, three or four times a week.

The effects of copper on the barnacle setting rate is being checked by submerging setting plates at each of the eight stations. These are counted and replaced every week or two, depending on the setting rate.

Air and water temperature checks are made each time a station is sampled. A salinity and turbidity determination is also made. These factors have quite an effect on the magnitude of the various parameters involved in the estimating of "normal" ecological conditions. Without them it would be difficult to account for seasonal fluctuations that occur and which might otherwise be attributed to fluctuations in copper concentration after the addition of the copper ore dike.

The lagoon is also analyzed for its naturally occurring copper. It is necessary to have this information in order to know the distribution pattern of copper that will originate from the ore dike.

All sampling is performed from a 14-foot outboard motor boat. The assumption is made that all samples collected at a station are taken from a homogeneously mixed body of water. To insure that this condition is so, 50 gallons of water is pumped by means of a stainless

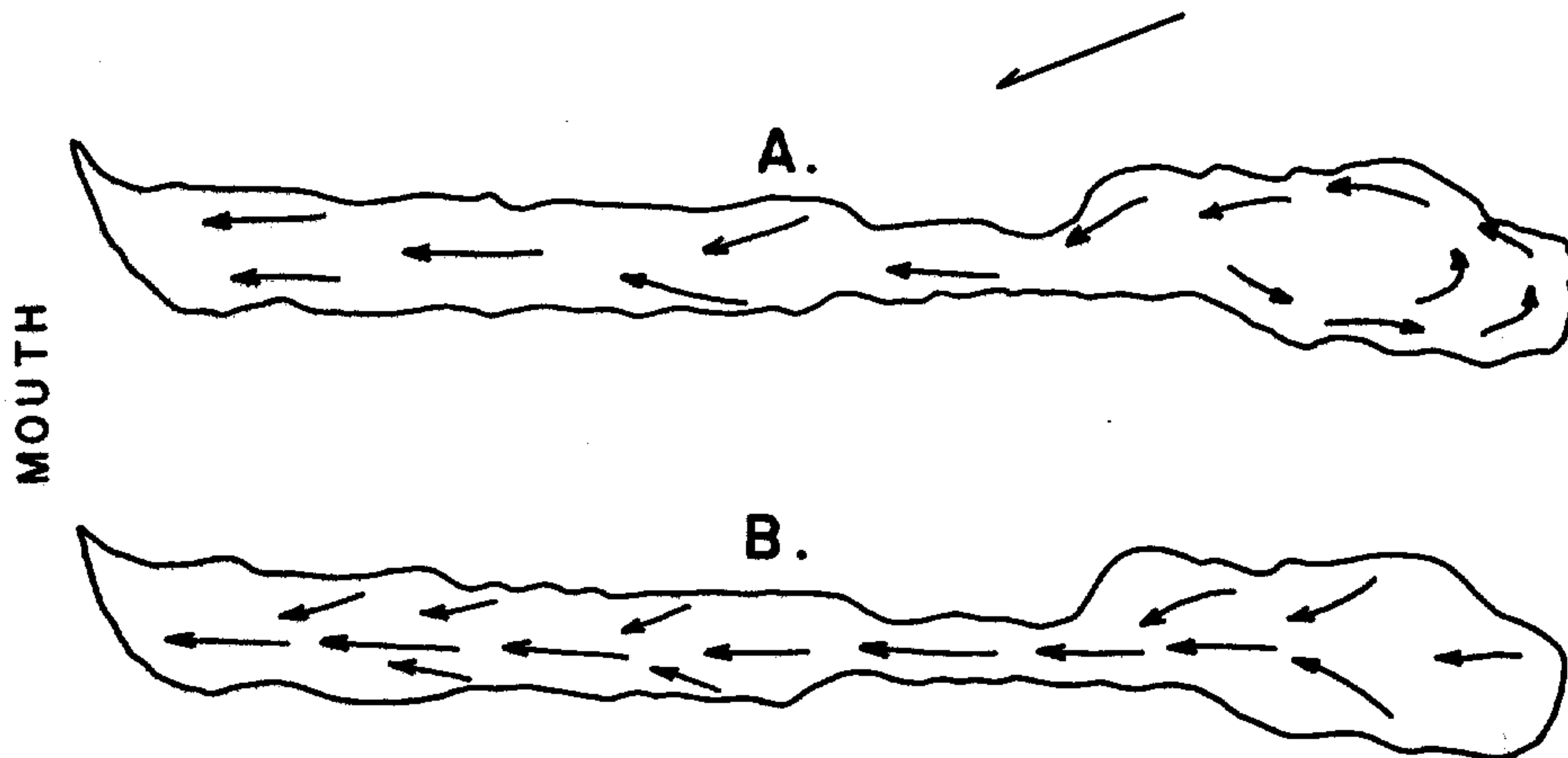


Collecting in East Beach Lagoon. Barnacle plates and flasks for zooplankton samples on bow, then plankton pump. Man aft is measuring turbidity with a Secchi disc. When taking *G. splendens* and chemical samples a polyethylene drum is carried on bow.

steel neoprene impeller type pump into a 53 gallon polyethylene tank. The water is pumped at a rate of 25 gallons a minute. This is fast enough to thoroughly mix the water. Samples are taken from this mixed volume of water from the bottom of the tank by means of a polyethylene hose outlet. An exception to this is the zooplankton sample. This is collected at the three locations mentioned by pumping 250 gallons of water through a number 10 plankton net.

It is necessary to have a measure of the reliability of the various analytical methods used in order to properly analyze the data. The ideal

way to obtain this would be to intersperse standard samples among the regular station samples. This would be difficult to do in this experiment, so an alternative method is employed. At unscheduled intervals, all samples are taken from the thoroughly mixed 53 gallons of water. These are then labeled and submitted to the analyst who analyzes them under the assumption that they are the regular station samples. The assumption is made that all samples taken from the drum are identical, therefore, the deviation between them supposedly representing the 8 stations is a reflection of the reliability of the analyses. This does not include systematic errors inherent in the analytical methods. However, since the majority of the conclusions of the experiment will be based upon differences, as has been explained, the absolute magnitudes are not too important.



Current patterns in East Beach Lagoon as determined by aerial photographs using fluorescein dye.

The surface current system of the lagoon has been determined by adding fluorescein during tide changes. The figures shows the surface current pattern after the start of an outgoing tide. A shows the current system half an hour after the start of an outgoing tide, and B, three hours later. A more exact estimate will be made by plotting copper concentration streaks after the addition of the ore dike.

Two lagoon parameters that have not shown excessive seasonal variation are chlorophyll and total zooplankton concentrations.

TOXICITY OF COPPER TO MARINE ORGANISMS

David V. Aldrich, Project Leader

A series of copper toxicity studies is being conducted to compare the sensitivity to copper poisoning of several organisms representative of the Galveston Island Lagoon. This type of information, gathered in the laboratory under controlled conditions, may be of value in the interpretation of population fluctuations encountered in the field observations which will form the largest part of the ore dyke experiment.

To date, the studies suggest that several Lagoon organisms may be arranged in this order of decreasing susceptibility to copper: Littorina, young sciaenids, young sparids, young mullet, grass shrimp (embryonated eggs as well as large and small adults).

The importance of water quality in modifying the toxicity of copper in fresh water is well recognized. Two experiments with the snail, Littorina irrorata, suggest that this is also true in sea water. In one case, 0.5 parts per million of copper in Lagoon water produced no retraction in test snails after 24 hours of exposure, while the same copper concentration in an artificial sea water or Florida coastal sea water produced retraction in 60 per cent and 90 per cent of the snails, respectively. Control snails, maintained in each of the three waters without copper, showed no retraction. In another experiment 60 per cent of the snails retracted within 24 hours in either the artificial sea water or Florida water with 0.5 parts per million of copper, whereas ten per cent retracted in Lagoon water with this copper concentration. In comparison, ten per cent of the snails retracted in the control (no copper) Florida sea water, and none retracted in the Lagoon water or artificial sea water controls. One hundred eighty snails were used in the two studies, ten in each experimental group.

In general, the Florida and artificial sea waters differ obviously from Lagoon water in containing little or no suspended matter. It may well be the absorptive surface provided by such suspended material that effectively reduces the number of free copper ions and reduces the apparent toxicity of copper in the latter medium.

To permit direct observations on the mortality of experimental animals in the presence of copper ore in non-stagnant sea water, a small-scale constant flow apparatus has been constructed. It has been set up in such a way that water can be separately and constantly passed through each of four 8-liter aquaria, two of which will serve as controls, the other two containing copper ore.

EFFECT OF INSECTICIDES ON MARINE ORGANISMS

Edward Chin, Project Leader

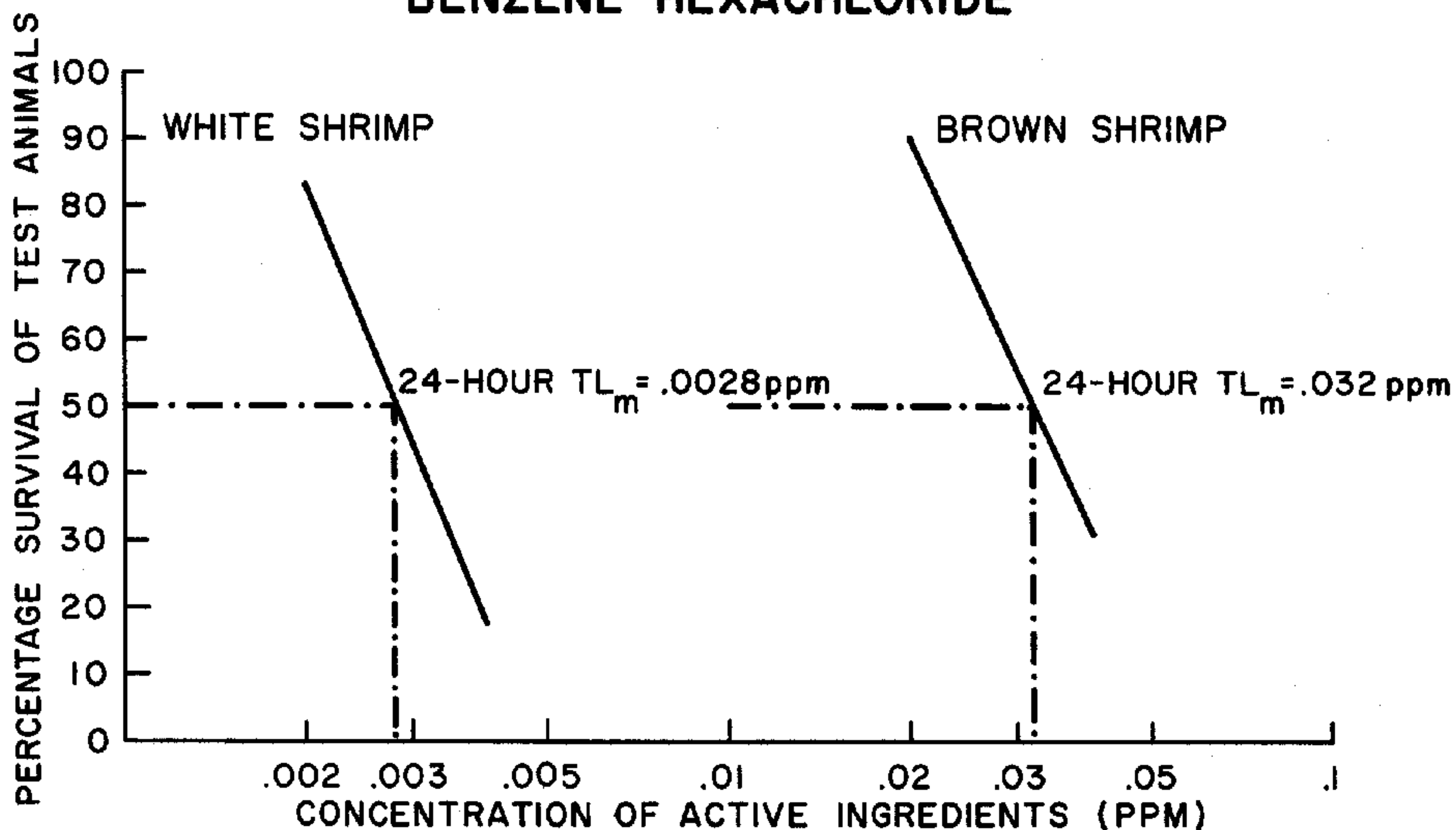
With the current campaign against fire ants in the southern United States, conservation officials and sportsmen are alarmed over the increasing use of such organic insecticides as dieldrin and heptachlor. The toxicity of these insecticides to fish and wildlife has been noted by many workers and caution in dispersing the material has been widely discussed. Previous work, however, has been largely on freshwater fishes, birds, and mammals. Earlier in the year, in response to requests by the Galveston County Mosquito Control group and local dealers in live bait shrimp, we investigated the effects on penaeid shrimp of a commercial insecticide containing 3.0 per cent gamma isomer and 5.1 per cent other isomers of benzene hexachloride. More recently we began studies to determine the relative toxicity of dieldrin and heptachlor to shrimp and other estuarine inhabitants.

Benzene hexachloride was tested on two size groups of shrimp, 11-13 mm and 29-50 mm in length. The smaller size group was composed of brown shrimp, Penaeus aztecus, while the larger size group was composed almost entirely of white shrimp, Penaeus setiferus. A dieldrin compound and a heptachlor compound, each containing 10 per cent active ingredients, were tested on mullet, Mugil cephalus.

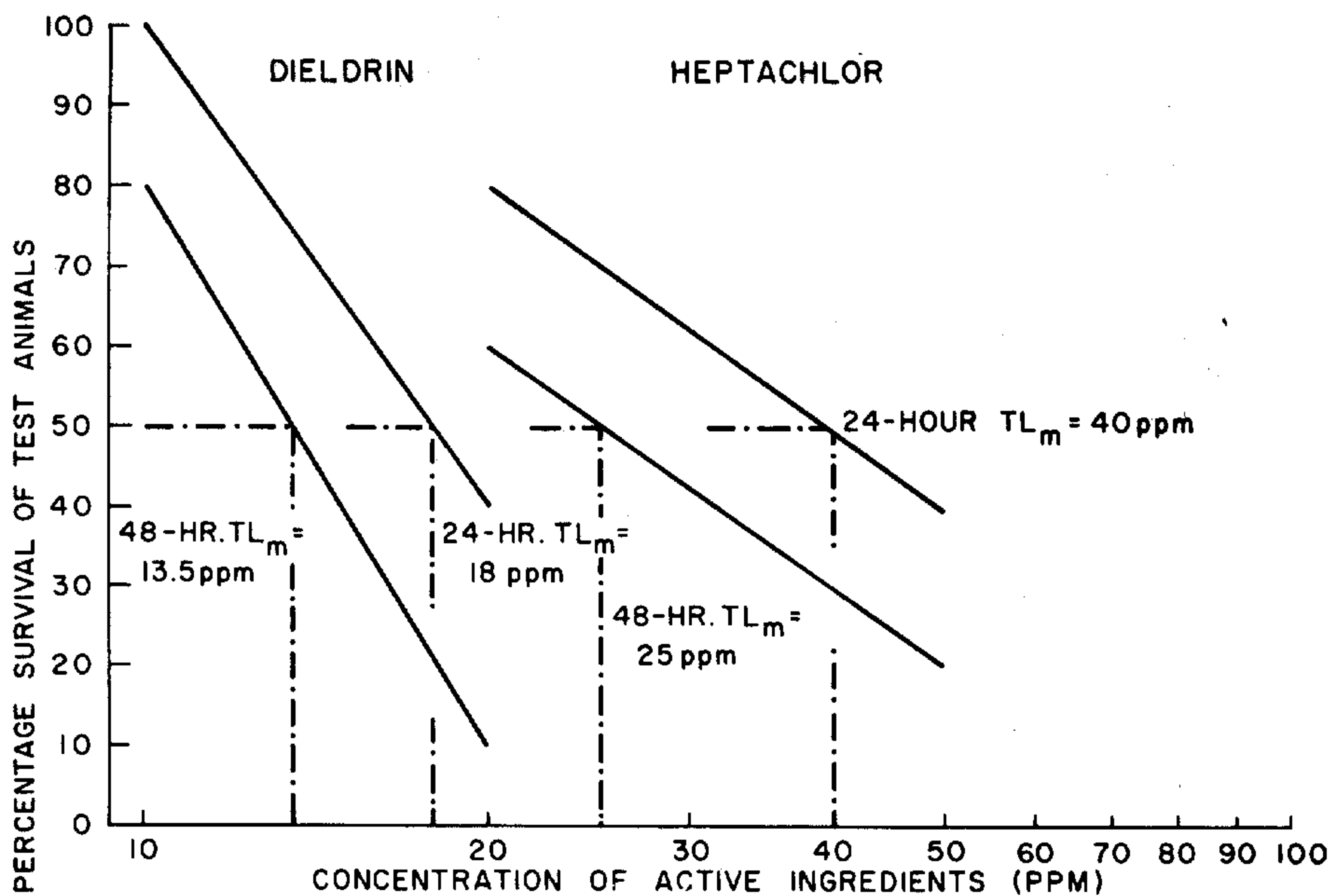
The median tolerance limit (TL_m) or the concentration at which 50 percent of the test animals are able to survive for a specified period of exposure was adopted to derive indices of relative toxicity.

Both size groups of shrimp were very sensitive to benzene hexachloride. Differences in sensitivity between the two groups were attributed to size rather than species differences. Mullet were more sensitive to dieldrin than heptachlor.

BENZENE HEXACHLORIDE



Toxicity of benzene hexachloride to brown and white shrimp.

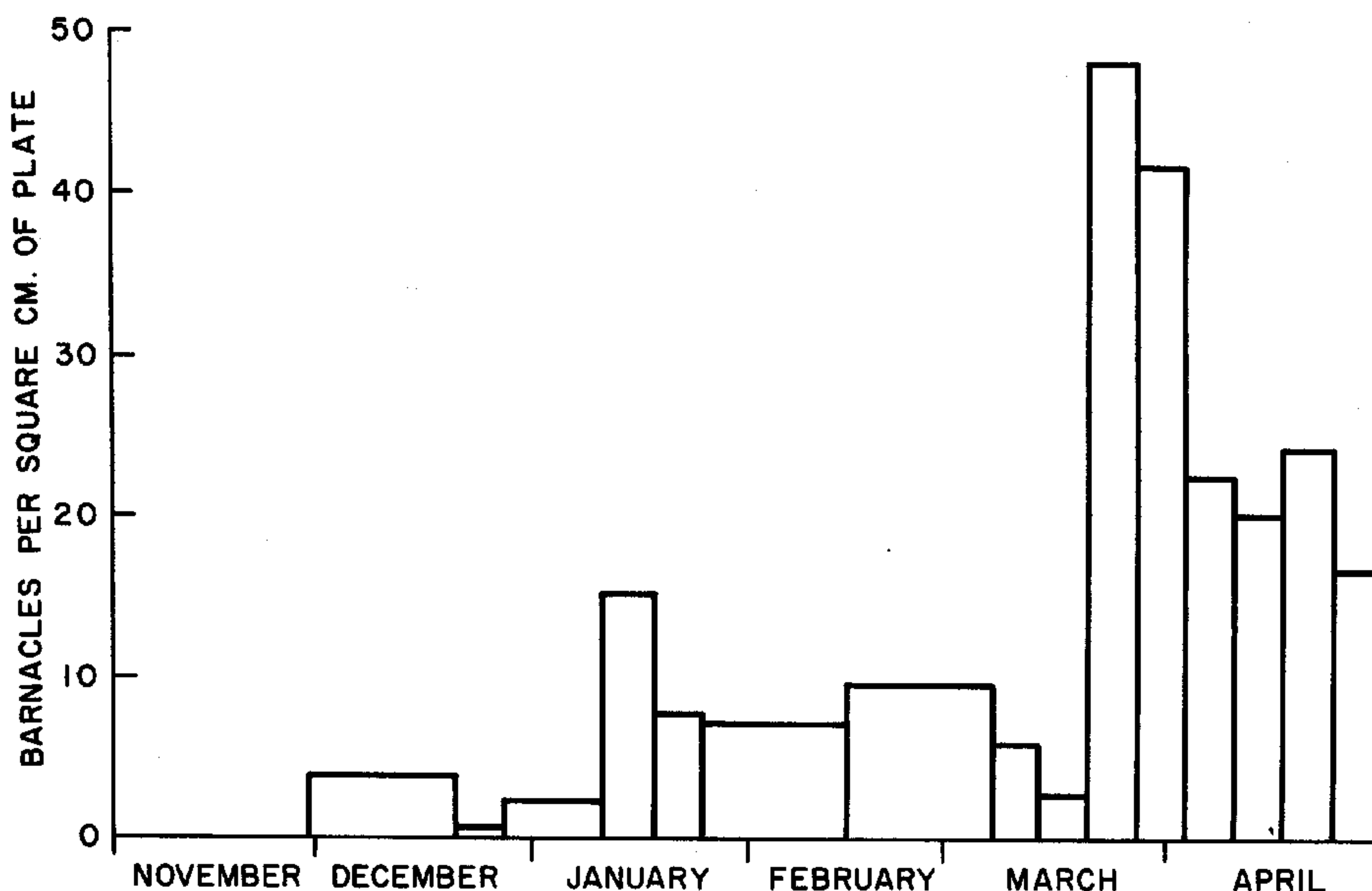


Toxicity of dieldrin and heptachlor to mullet, Mugil cephalus.

BARNACLE ATTACHMENT RATES AT GALVESTON, TEXAS

David V. Aldrich, Project Leader

As part of the population studies in the Galveston Island East Beach Lagoon, barnacle attachment rates were measured from October, 1957 to date by the fouling plate method of Butler (1955). Part of the seasonal cycle is reflected in the weekly averages of attachment rate as shown in the figure.



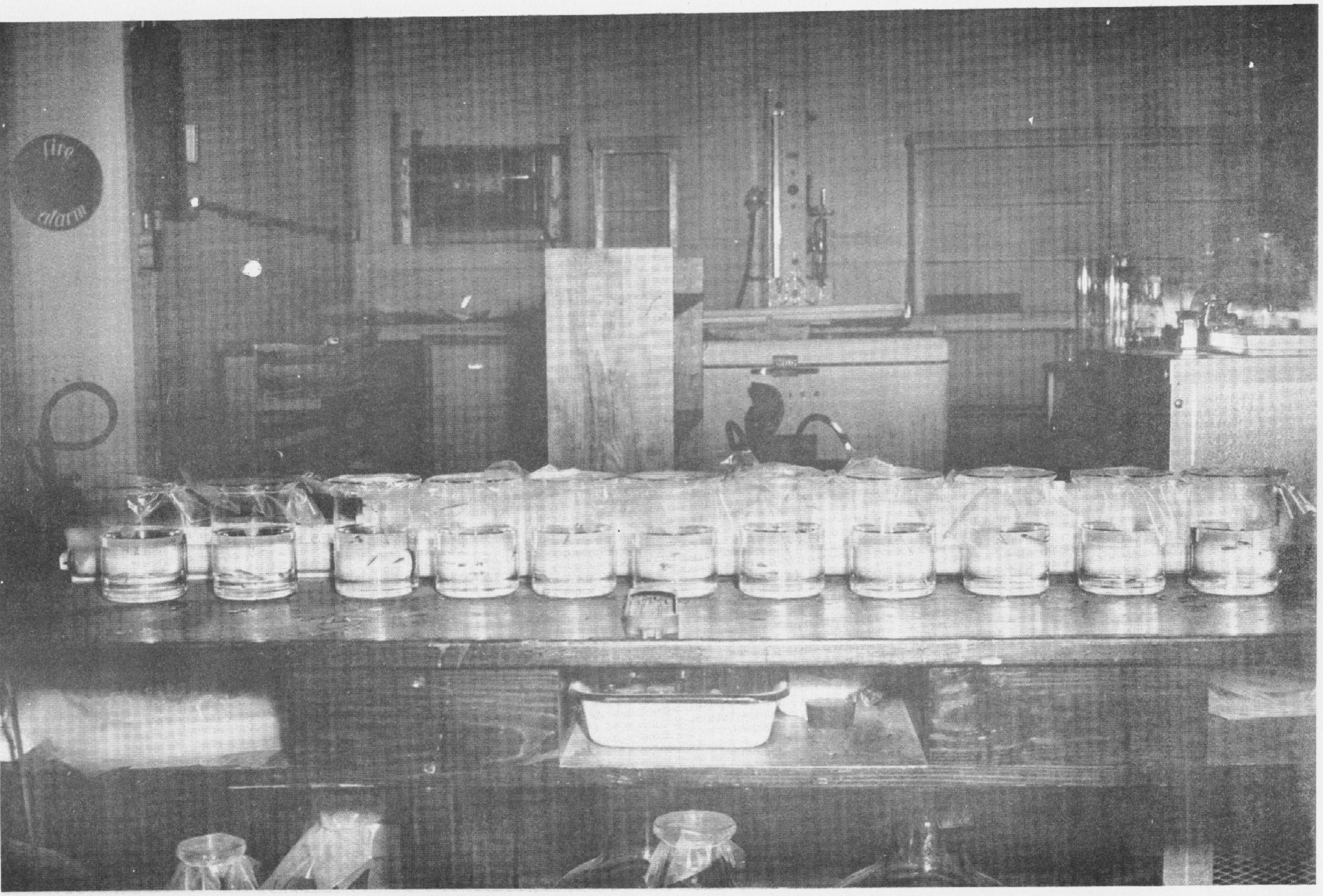
Average barnacle attachment for eight stations in East Beach Lagoon, Galveston, Texas, November 1957 through April 1958.

These population estimates will be continued in an effort to determine the complete seasonal cycle of barnacle attachment under "normal" environmental conditions. Upon the subsequent introduction of a copper ore dyke into the Lagoon, continuing counts should assist in the evaluation of immediate effects in terms of barnacle attachment rate.

TOXICITY OF GONYAULAX MONILATA TO FISH

Jean Gates, Technical Aide

Experiments conducted during the past year demonstrated that bacterized cultures of Gonyaulax monilata are toxic to fish. Experiments using mullet (Mugil cephalus) and guppies (Lebistes reticulatus) as test animals show that a culture of G. monilata containing approximately 1.4 million organisms per liter was lethal in approximately four hours or less. G. monilata has been associated with fish mortality near Melbourne, Florida and near Galveston, Texas on several occasions. The table is a summary of the results of one experiment conducted with G. monilata. Previous investigators consider the mortality of fish associated with these blooms to be caused by oxygen deficiencies resulting from decomposition of the organisms of the bloom. Suboptimal oxygen content may occur, but the fact that the cultures are toxic in laboratory tests emphasizes the probability that toxins are produced by these natural blooms. The figure shows the experimental arrangement used in these tests.



Experimental arrangement of materials for tests of Gonyaulax monilata toxicity to mullet (Mugil cephalus).

TOXICITY OF THE DINOFLAGELLATE, Gonyaulax monilata TO STRIPED MULLET, Mugil cephalus

Material Tested	Distress Time (Range for 6 fish in Hrs:Min)	Death Time (Range for 6 fish in Hrs:Min)	Remarks
<u>G. monilata</u> culture--heated to 50° C and cooled to room temperature.	0:31-0:42	0:36-0:58	
Culture medium--uninoculated, heated to 50° C and cooled to room remperature.	None	None	All fish lived 24 hours test period.
<u>G. monilata</u> culture--frozen 24 hours and heated to room temperature.	0:47-0:51	0:50-1:00	
Culture medium--uninoculated frozen 24 hours and heated to room temperature.	None	None	All fish lived 24 hours test period.
Millipore filtrate of <u>G. monilata</u> culture.	1:22-3:04	1:27-3:24	
Millipore filtrate of culture medium--uninoculated.	None	None	All fish lived 24 hours test period.
Millipore residues of <u>G. monilata</u> culture reconstituted in culture medium.	None	None	

TOXICITY OF THE DINOFLAGELLATE, Gonyaulax monilata TO STRIPED MULLET, Mugil cephalus

(Cont'd)

Material Tested	Distress Time (Range for 6 fish in Hrs:Min)	Death Time (Range for 6 fish in Hrs:Min)	Remarks
<u>G. monilata</u> culture--unaltered.	1:55-3:09	2:04-4:03	
Culture medium--uninoculated and unaltered.	None	None	All fish lived 24 hours test period.
<u>G. monilata</u> culture--unaltered aerated.	2:15-3:22	2:34-4:25	
Culture medium--unaltered aerated.	None	None	All fish lived 24 hours test period.

HISTOLOGICAL TECHNIQUES FOR GYMNOINIUM SPP.

Dave V. Aldrich, Project Leader

Since histochemistry usually provides the best first approach to chemical information concerning the cellular constituents and activities of Protozoa, attention has been directed toward the problem of fixing Gymnodinium breve. Successful preservation may also be of value in certain field population studies, in which prolonged handling of large numbers of the delicate living organisms before counting may be undesirable.

The fragility of G. breve is clearly indicated by results obtained with usual histological fixatives. Formalin, Bouin's, Zenker's, and Schaudinn's fluids yielded only a very occasional recognizable cell. Alcohol, acetone, and trichloroacetic acid also left few intact organisms, and iodine vapor, Lugol's solution, and a mixture of chromic and acetic acids were only slightly better. Best results have been obtained with 0.5 cc of 1% osmic acid per 10 cc. of G. breve culture. This method will best preserve the living appearance of the organism, yielding about 70 percent of the live cells in recognizable condition.

CHLORINITY SAMPLING AND STORAGE

Kenneth T. Marvin, Project Coordinator

In an effort to demonstrate the importance of placing more emphasis upon the improvement of collection and storage of salinity samples, the marine laboratories of the Gulf Fishery Investigations and the Texas Agricultural and Mechanical College have jointly conducted experiments designed to check the effectiveness of one of the commonly used methods for the collection and storage of salinity samples. The experiments demonstrated the extent to which systematic errors limit the reliability of an analysis and also show that the greater portion of these errors believed to be due to evaporation, can be greatly minimized by the proper sealing of sample closures.

The experiments also demonstrate the possibility of developing systematic errors through use of improperly controlled substandard or secondary standard sea water standards.

An analysis of the data collected was made to demonstrate that real differences account for much of the variation noted between duplicate samples.

The samples were collected at three stations located in the Gulf of Mexico. All samples were divided as to station and depth and sent to the two laboratories for analyses. Interspersed among the station samples were laboratory prepared control samples.

Both laboratories employed the Mohr-Knudsen method of chlorinity analysis. End points were detected electrometrically. Calibration of the silver nitrate reagent was maintained in one of the laboratories by running several secondary standard sea water checks prior to the day's session, and in the second by running a Copenhagen standard after each fifth sample analysis.

The results of the analyses showed that a systematic difference of more than .05 parts per thousand as salinity existed between the results of the two laboratories. About two thirds of this was traced to sample evaporation during storage. The other third could not be definitely accounted for.

Another interesting observation was that replicate analyses of samples taken from the Nansen bottles at one of the three stations resulted in significantly higher variation than at the other two. This fact indicates that real differences in chlorinity can exist within very small volumes of water.

PHOSPHORUS CONTENT OF MARINE ORGANISMS

Kenneth T. Marvin, Project Coordinator

and

Larence M. Lansford, Chemistry Analyst

This project was commenced several years ago and is being continued as time permits. The fish and shrimp specimens included in this study were taken from the Galveston, Texas coastal and bay waters. The one exception is an unidentified species of lantern fish (family Myctophidae) which was taken about 50 miles southeast of the Mississippi Delta. The analyses are based on the whole animal. This fact, of necessity, limits the specimen size.

All samples were prepared by wet ashing with sulphuric and nitric acids. They were then diluted sufficiently to bring the phosphorus concentration to within a suitable range to be analysed using our regular sea water phosphate method. Some of these results are shown in the table.

For 1953 the Fishery Statistics of the United States show that 505,794,000 pounds of fish and 224,504,000 pounds of shrimp were caught in the Gulf of Mexico. If we assume that the data in the table approximates the mean phosphorus content for this catch, this would be equivalent to the removal from the Gulf of Mexico fishing areas of approximately 4 1/3 million pounds of phosphorus. In terms of conventional units, this amount would be sufficient to raise the phosphorus concentration level of an area of 3,000 square miles having a depth of 50 feet by 0.53 microgram atoms of phosphorus per liter. This amount is considerably above the average concentration of phosphorus found in waters of this depth in the Gulf of Mexico.

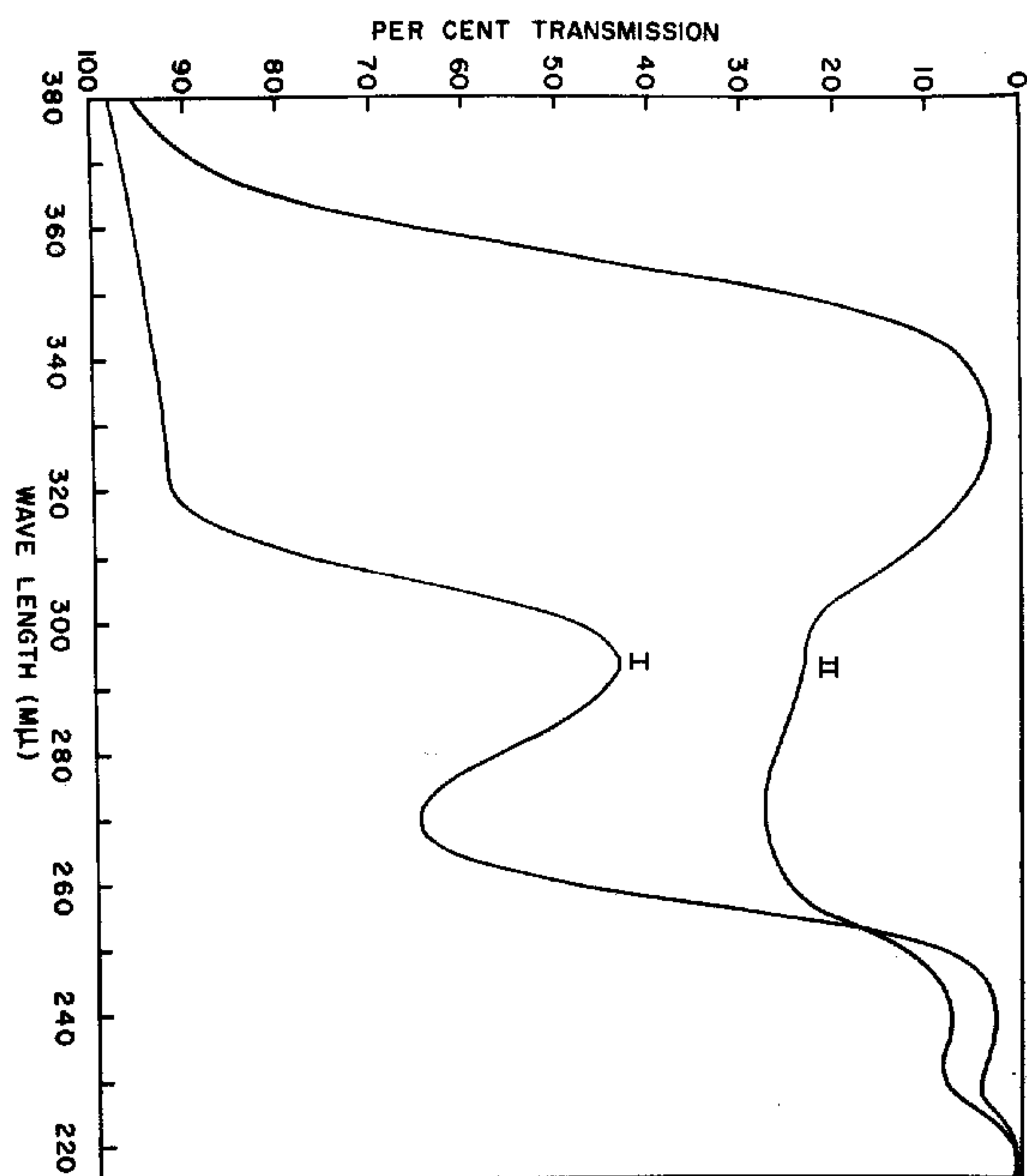
Species	N.	Ave. Whole Weight (g.)	% Phosphorus	
			Dry Wr. Basis	Whole Wt. Basis
Lantern Fish (Undetermined species of family Myctophidae)	40	1.2	3.11	0.82
Broad Killifish <u>Cyprinodon variegatus</u>	24	1.1	3.08	0.70
Ling <u>Phycis floridanus</u>	22	46.8	2.43	0.47
Anchovy <u>Anchoviella mitchilli</u>	19	0.3	2.91	0.66
Mullet <u>Mugil cephalus</u>	16	20.5	3.31	0.83
Menhaden <u>Brevoortia patronus</u>	14	27.0	3.39	0.80
Shrimp--Male <u>Penaeus setiferus</u>	7	22.7	1.49	0.36
Shrimp--Female <u>Penaeus setiferus</u>	18	23.7	1.41	0.34

DETERMINATION OF ORGANIC COMPOUNDS IN SEA WATER

Zoula P. Zein-Eldin and Billie Z. May, Chemists

This work was begun several years ago, and is largely complete. Two types of compounds, carbohydrates and proteins, were of major interest both because of their possible importance to marine organisms as nutritional sources and because of their use as indicators of productivity of the water itself. Thus, it was necessary to find methods which could be used routinely for the measurement of each type of compound in the sea water. The Erdman method for the determination of carbohydrates with N-ethyl carbazole had been used successfully both here and in other laboratories, but it was felt to be somewhat insensitive. Thus, the method was exhaustively restudied in an attempt to improve both the sensitivity and the accuracy of the method. It was found that this could be done if the reagent was added in two steps rather than in one as originally suggested by Erdman. This has resulted in a new method (more extensively treated in a paper now submitted for publication) which is sensitive to as little as 1 mg. of carbohydrate per liter of sea water.

We have also been interested in the determination of proteins in sea water. We have found that tyrosine, an aromatic amino acid present in all proteins, undergoes certain structural changes when it is heated under pressure in the presence of alkali and salt. These structural changes which are reflected in differences in the absorption spectrum of the compound after autoclaving, have been used as the basis of a new method for the determination of tyrosine and protein in sea water.



Curve I shows tyrosine +0.175N NaOH

Curve II shows the same autoclaved for 3 hours at 80 pounds pressure.

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